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SCHOOL OF MEDICINE
UNIVERSITY OF MIAMI

Department of Dermatology and Cutaneous Surgery
Wound Healing Research Laboratory

Pilot Study Report

Determination of the Debridement Effects of Revity on Deep Dermal Wounds in a Porcine Model

December 10, 2021
(January 15, 2022 revised)

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INSTITUTIONAL POLICIES AND REGULATIONS

The following experiment was submitted for approval by University of Miami's Animal Use Committee. This study was conducted in compliance of the University of Miami's Department of Dermatology & Cutaneous Surgery's Standard Operating Procedures (SOPs). Animal was monitored daily for any observable signs of pain or discomfort. In order to help minimize possible discomfort, two analgesics (buprenorphine and fentanyl transdermal) were used.

OBJECTIVE

The objective of this study is to evaluate Revity using a deep dermal wound model.

Study Endpoints

The primary endpoint of the study to assess the capability of the treatments to remove slough on deep dermal wounds. Quantifying the amount of slough removed using Image J and determine the amount of Methicillin Resistant *Staphylococcus aureus* (USA300) removed from the wound bed. The secondary endpoint of this study is to determine the potential treatment response of the treatments on the healing.

MATERIALS AND METHODS

Experimental Animals

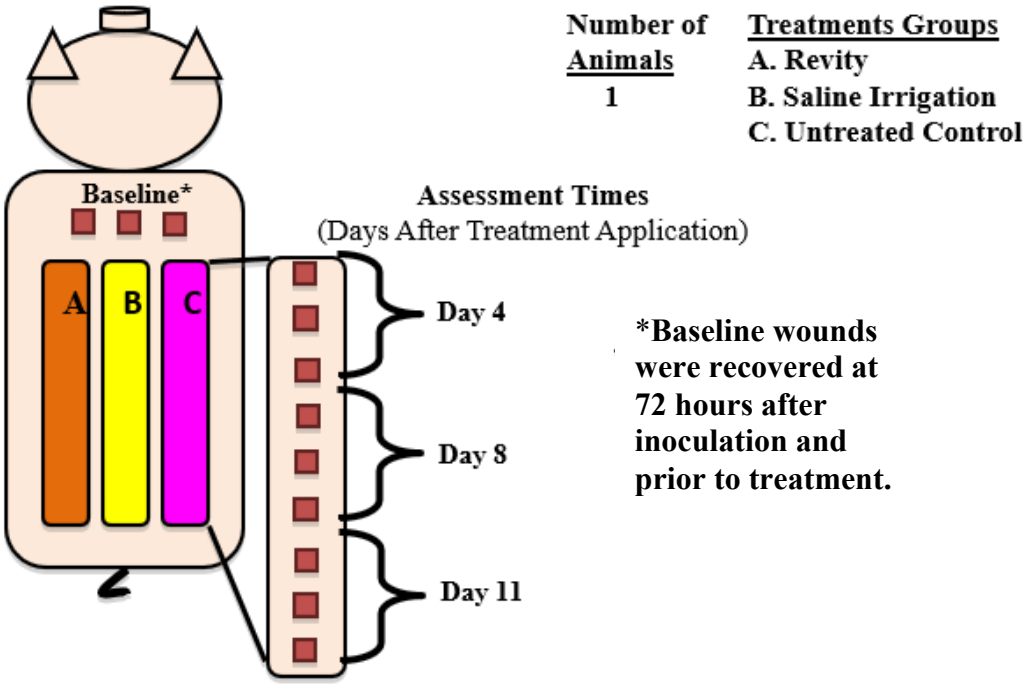
A porcine model was used for our experimental research due to the morphological similarities between swine skin and human skin. [1] One (1) animal was used for this study. The young specific pathogen free (SPF: Looper Farms, North Carolina) pig weighing 35-45 kg was kept in house for at least 5 days prior to initiating the experiment. The animal was fed a basal diet *ad libitum* and housed individually in our animal facilities (meeting American Association for Accreditation of Laboratory Animal Care [AAALAC] accredited) with controlled temperature (19-21^{o}C) and lighting (12h/12h LD).

Wounding Technique

The back of the experimental animal was clipped with standard animal clippers on the day of the experiment. The skin on both sides of the animal was prepared for wounding by washing with a non-antibiotic soap (Neutrogena Soap Bar; Johnson and Johnson, Los Angeles, CA) and sterile water. Each animal was anesthetized and given analgesics till the end of the study.

Thirty (30) deep reticular dermal wounds measuring (22 mm x 22 mm x 3 mm deep) were made in the paravertebral and thoracic area with a specialized electrokeratome fitted with a 22 mm blade (see Appendix 1 for timeline). The wounds were separated from one another by 5-7 cm of unwounded skin. All wounds were inoculated within 20 minutes after wounding (see Wound Inoculation below). On Day 0 (after 72 hours biofilm formation), three (3) wounds were recovered as described below for baseline counts. The other twenty-seven (27) wounds were randomly divided into three (3) treatment groups with nine wounds according to the experimental design below (Figure 1).

Figure 1: Experimental Design



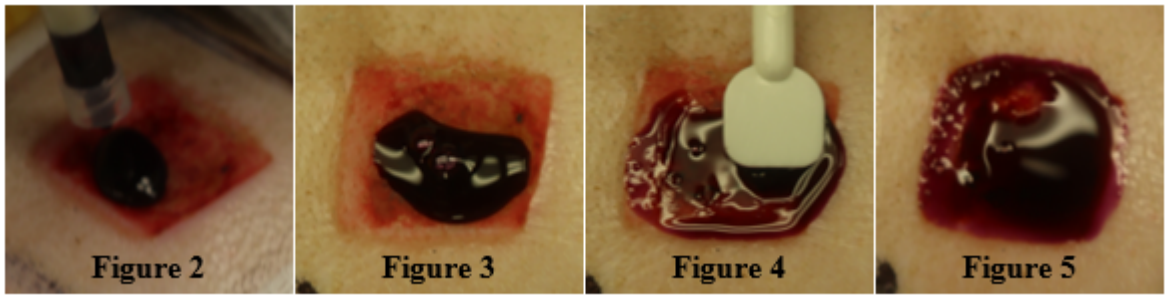
Wound Inoculation

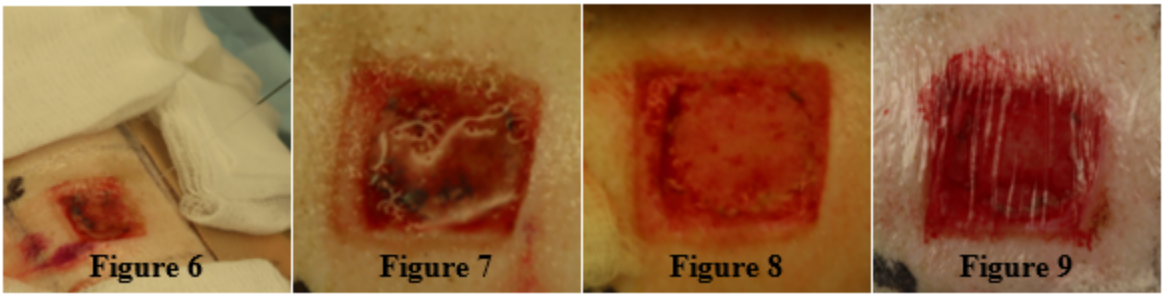
A pathogenic strain of Methicillin Resistant *Staphylococcus aureus* (USA300) was used in this study. All bacterial inoculum suspensions were made by swabbing a 3-cm diameter area of the overnight growth from a culture plate into 4.5 mL of sterile water. This resulted in a suspension consisting of approximately 10^{10} colony forming units/mL (CFU/mL). One mL of this suspension was diluted into 35 mL of Tryptic Soy Broth (TSB), making the inoculum suspension 10^6 CFU/mL. A sample of this suspension was further diluted and plated onto culture media to enumerate viable CFU/mL of organism prior to the experiment. The inoculum suspension was used directly to inoculate each wound by pipetting a 25 μL aliquot into the center of each wound site within 20 minutes after wounding. The inoculum was scrubbed into the wound site with a sterile spatula for 30 seconds. All wounds were covered with a polyurethane film dressing (Tegaderm Transparent Dressing; 3M Health Care, St. Paul, MN USA) for 72 hours to allow for slough and biofilm formation.[2] Dressings were secured with surgical tape and wrapped with Coban elastic wrap (3M, St. Paul MN).

Treatment Regimen

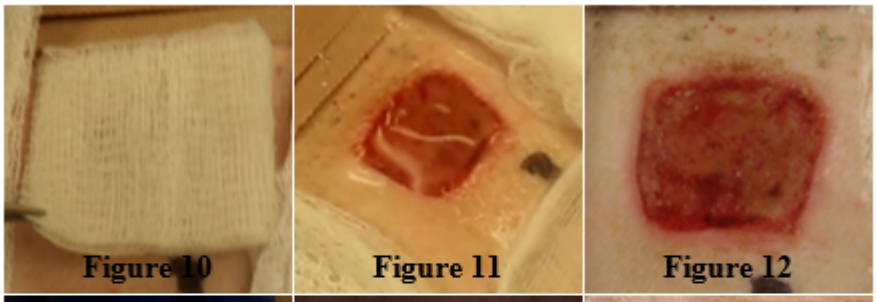
After 72 hours after wounding and infection (Day 0 of treatment) the Tegaderm dressings were removed, and 3 wounds were recovered as a baseline. The remaining wounds were treated with one of the following treatments groups: A) Revity, B) Saline, or C) Untreated Control.

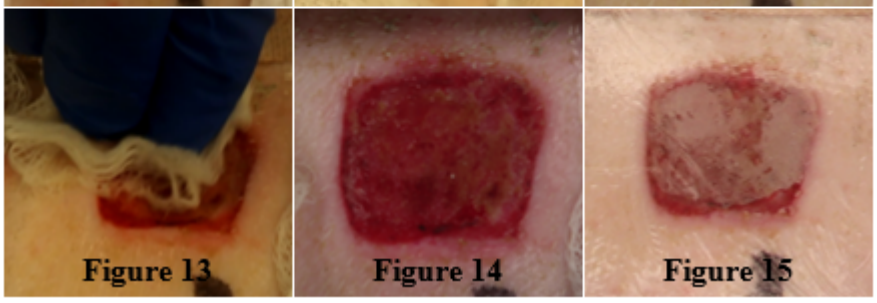
The first treatment group had each wound receive 500 μL of Revity (see Figures 2 and 3) which was spread with a sterile spatula and allowed to stay in place for 30 seconds (Figures 4 and 5). After 30 seconds, all wounds were rinsed with a 10mL syringe of sterile saline (Figures 6 and 7), then gently wipe with moistened sterile PBS gauze (Figure 8) and then covered with Tegaderm as shown in Figure 9.





Saline Irrigation wounds each had a premoisten gauze (500 µL of sterile saline) placed over the wound which was allowed to stay in place for 30 seconds (Figure 10). All these wounds were rinsed (Figures 11 and 12) and gently wipe with moistened sterile PBS gauze (Figures 13 and 14) as done above after the 30 seconds. These wounds were then covered with Tegaderm as seen in Figure 15.





Untreated Control wounds were rinsed with a 10mL syringe of sterile saline (Figure 16) and then gently wipe with moistened sterile PBS gauze (Figures 17 and 18), then covered with Tegaderm dressings as shown in Figure 19.

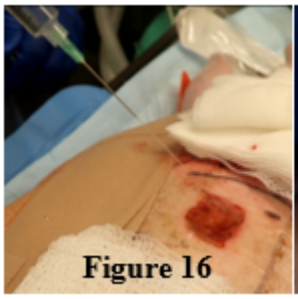


Figure 16

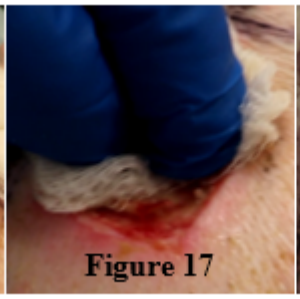


Figure 17

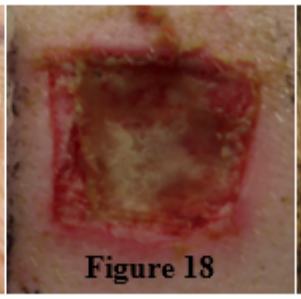


Figure 18

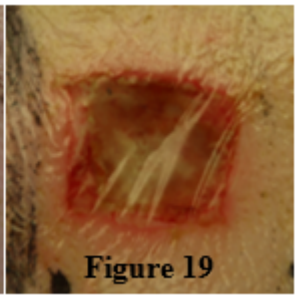


Figure 19

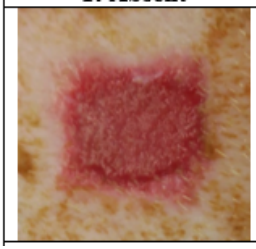
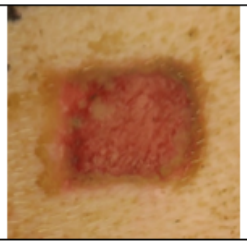
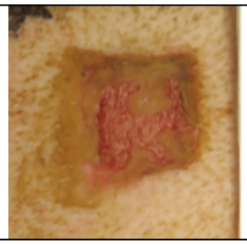
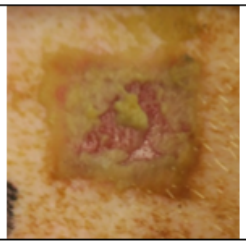
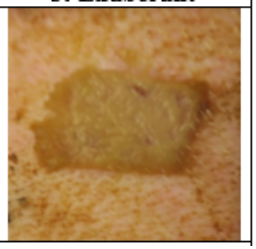
All treatments were applied only once and all Tegaderm dressings were secured in place with tape and covered with Coban wrap (3M, St. Paul MN).

Clinical Observations

The amount of slough remaining was observed as seen above by gently wiping each wound, the slough was score using the scale below on Figure 20, Appendix 1 shows the raw data on Table 1 for slough observations.

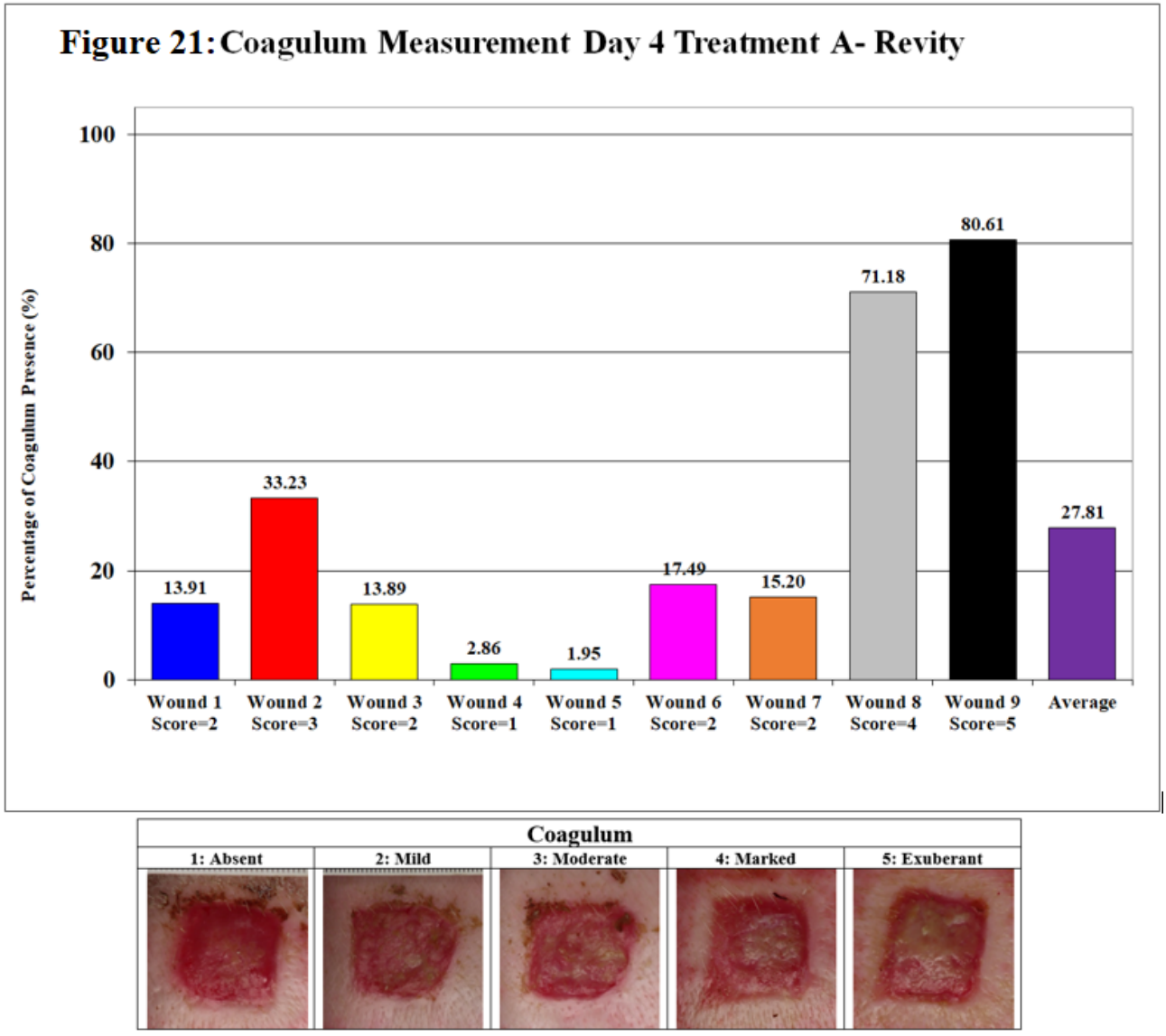
Slough – degree of moist devitalized tissue*
* Score: 1 = absent, 2 = mild, 3 = moderate, 4 = marked, 5 = exuberant

Figure 20:
Slough Scores

1: Absent	2: Mild	3: Moderate	4: Marked	5: Exuberant
				

Coagulum – visual observation of greyish over the wounds*
* Score: 1 = absent, 2 = mild, 3 = moderate, 4 = marked, 5 = exuberant

Coagulum scores were performed only for Revity treated wounds on Day 4, no visual presences of coagulum were observed on other days and/or other treatment groups. The raw data for all these wounds can be found below in Figure 21, Appendix 1: Table 2, and Figure A.



Wounds being treated with Revity on day 4, exhibited different degrees of coagulum. Wound #2 was slightly higher at 33.23% (score 3), while wounds #8 (score 4) and #9 (score 5) showed substantially higher coagulum percentages with 97.18 and 80.61%, respectively. Because of these outliers, then the average percentage (27.87%) could be impacted with a value higher than anticipated by observing the remaining wounds. It should also be mentioned that wounds #4 and #5 with a score of 1 exhibited small traces of coagulum on their respective wound beds. However, wounds #1, 3, 6 and #7 with a score of 2, showed 13.91, 13.89, 17.49 and 15.20, respectively. 2.86 and 1.95%, as shown in Figure 21.

Erythema (redness)* – indicative of the amount of inflammation present*
* Score: 1 = absent, 2 = mild, 3 = moderate, 4 = marked, 5 = exuberant

On Day 0 (after 72 hours of biofilm formation), all wounds in each treatment group had mild erythema. On Day 4 till the end of the study there was no erythema observed on all the wounds (Appendix 2: Figure B).

Digital Photography & Measurement of the Slough Removal

Photographs were taken before and after treatment by using two rulers that were placed tangential on the wounds, these photos were sized to scale. The wound area that includes slough was traced by digital imaging with ImageJ. In addition, the areas that clinically appear to show removal of slough/coagulum were also digital traced to determine potential debridement effects of the treatments (see Figure 22: photos from a burn study showing slough/eschar measurements).

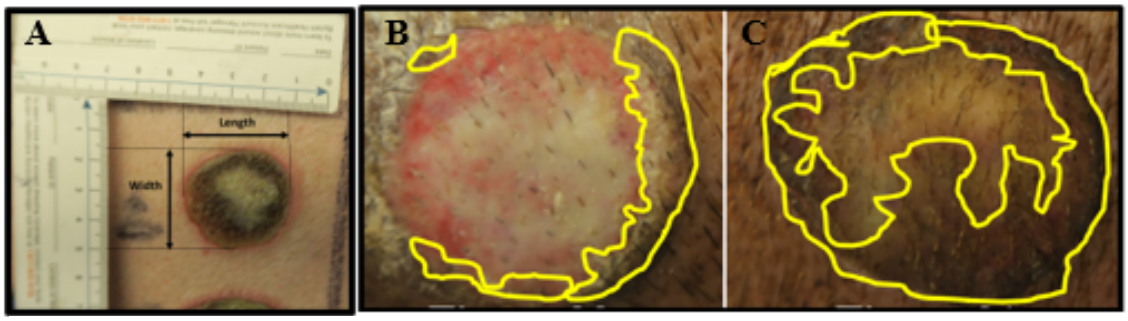


Figure 22: Scaling of Photograph (**A**) and measurement of slough removal [before (**B**) and after (**C**)].

Microbiology Assessment

On Day 0 (72 hours after wound inoculation) three wounds were biopsied (6mm punch biopsy – see Figure 23 ➔) to obtain baseline counts prior to treatment. An incisional biopsy was also taken.

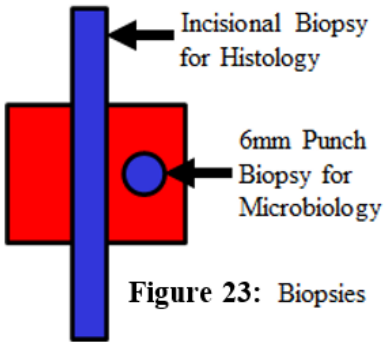
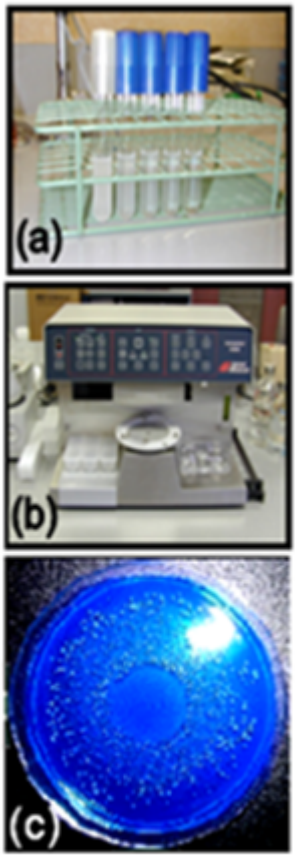


Figure 23: Biopsies

On Days 4, 8 and 11 (after treatment application and clinical observation) three wounds were also biopsied for microbiology. The microbiology biopsies (6mm) were weighed and immediately placed in 1 mL of All Purpose Neutralizing Solution. The sample was combined with an additional 4 mL of Neutralizing Solution and homogenized in a sterile homogenization tube. Serial dilutions (Figure 24 photo **a**) were made from all culture samples and the extent of microbiological contamination assessed using the Spiral Plater System (Spiral Biotech, Norwood, MA – Figure 24 photo **b**). This system deposits a 50µL aliquot of the scrub bacterial suspension over the surface of a rotating agar plate. Oxacillin Resistance Screening Agar (ORSAB) was used to isolate MRSA USA300 (Figure 24 photo **c**). All plates were incubated aerobically overnight (24 hours) at 37°C, after which the number of viable colonies were counted. This method has been used for over 34 years to evaluate the antimicrobial efficacy of various topical agents and/or dressings. [3,4,5,6,7,8,9,10]

Figure 24:



(a) Serial dilutions
(b) Spiral Plater
(c) Selective media

Histological Assessment

Three wounds were biopsied on Days 4, 8 and 11 post treatment. The incisional biopsy was obtained through the center of the wounds including normal adjacent skin on both sides (see Figure 23 above). These specimens were placed in formalin then stained with hematoxylin and eosin (H&E). One section per block was analyzed. The specimens were evaluated blinded via light microscopy and examined for the following elements of to determine a potential treatment response.[11]

1. Percent of wound epithelialized (%). Measurement of the length of the wound surface that has been covered with epithelium.

2. Epithelial thickness (cell layers μm). The epithelial thickness may vary from area to area within the biopsy. The thickness of the epithelium in μm was measured on five equal distance points from each other in the biopsy and averaged.

3. White cell infiltrate. Measured by the presence and amount of subepithelial mixed leukocytic infiltrates. Mean Score: 1 = absent, 2 = mild, 3 = moderate, 4 = marked, 5 = exuberant.

4. Granulation Tissue Formation. The approximate amount of new granulation tissue formation (dermis) was graded as follows: 0 = 0, 0.5 = 1-10%, 1 = 11-30%, 2 = 31-50%, 3 = 51-70%, 4 = 71-90%, 5= 91-100%

5. New Blood Vessel Formation: Presence of new blood vessels (non-quantitative). Mean Score: 1 = absent, 2 = mild, 3 = moderate, 4 = marked, 5 = exuberant.

Molecular Assessment (real-time RT-PCR)

From the previous study, a total of 4 mm punch biopies were taken from each wound. The biopsies were immediately submerged in RNAlater stabilization solution and incubated at 4°C overnight before being stored at -20°C. A total of 30 samples were assessed.

RNA was extracted and purified from collected porcine skin wound biopsies stored in RNAlater using Direct-zol RNA Extraction Kit (ZYMO Research) following manufacturer's instructions. In-column DNase I digestion was carried out to eliminate genomic DNA contamination. Real-time qPCR was carried out using One-Step RT-PCR Kit (Quanta Biosciences Inc.) to assess gene expression of IL-1α, IL-6, MMP-1, MMP-9, and TNFα.

For each reaction, 10 ng of total RNA was used as template. Real-time qPCR was performed in triplicates using the CFX96 real-time PCR system (Bio-Rad). Relative expression was normalized to the internal control GAPDH, and plotted as mean of fold changes ± SEM. Statistical analysis was performed to determine whether changes in the levels of gene expression are statistically significant ($p<0.05$).

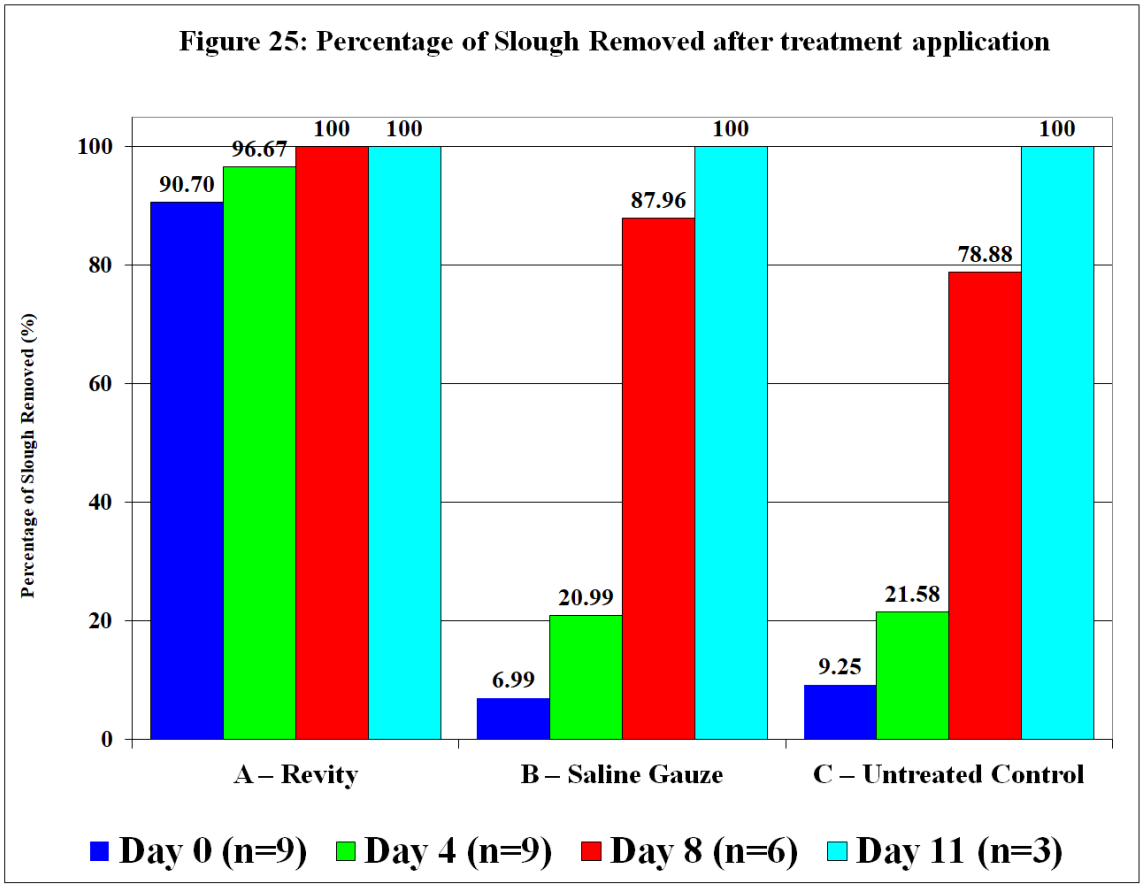
RESULTS

After treatment wounds with treated with Revity showed some punctate bleeding during removal (Photo C in Appendix 2 below). The other treatment groups had no bleeding reaction after their regimen.

Slough Scoring

Slough was scored to determine any potential debridement caused by the treatments. When comparing treatment groups, those wounds treated with Revity showed the highest percentage of slough removal on every assessment day (Figure 25). These wounds exhibited 90.70% of slough removed as early as day 0. While slightly increasing to 96.67% on day 4 and then reaching a full 100% from day 8 until the end of the study (day 11). Saline Gauze was only able to remove 6.99% of slough by day 0 and then almost triple the results (20.99%) on day 4. There was a substantial increase from day 4 to day 8, showing a slough percentage of 87.96, and subsequently reaching 100% on day 11.

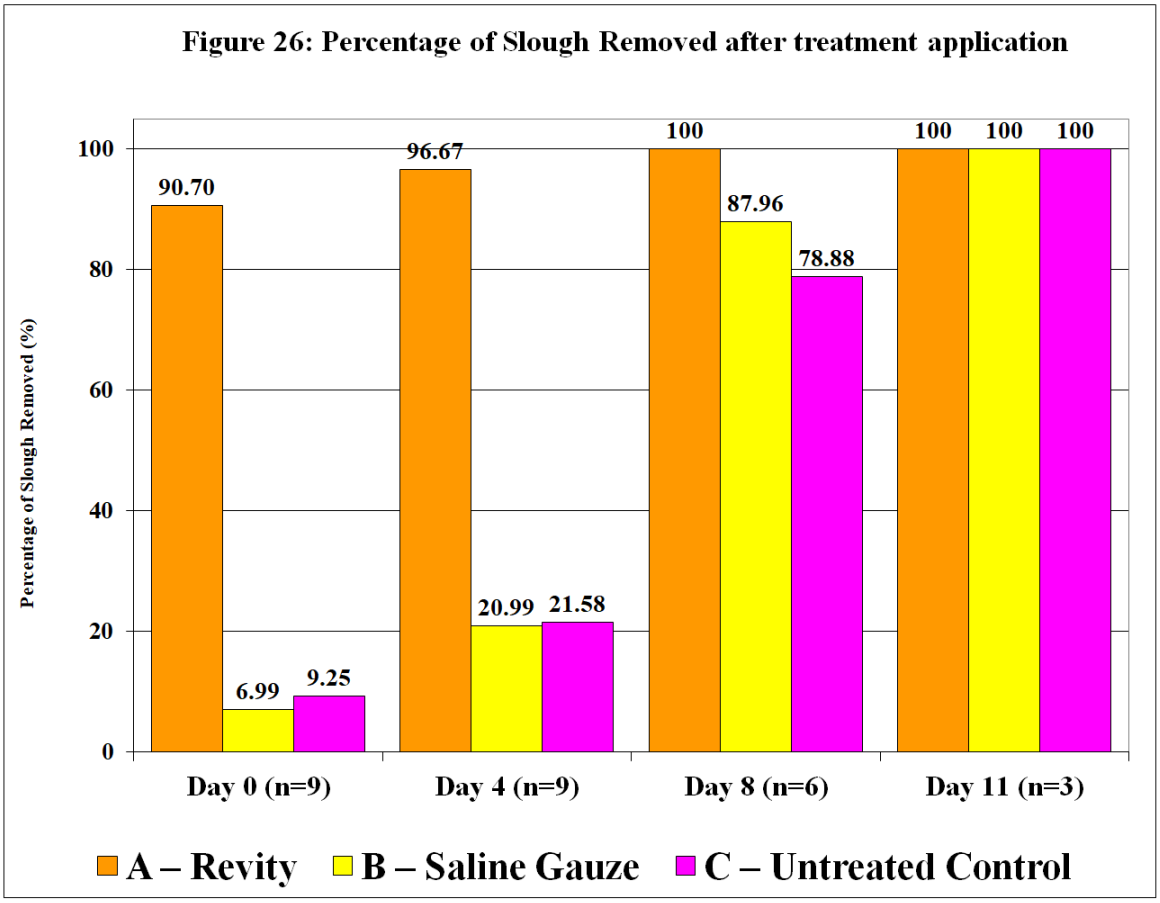
Figure 25: Percentage of Slough Removed after treatment application



Those wounds left untreated showed slightly different results than those wounds treated with Saline Gauze on every time point. On day 0, Untreated Control had a 9.25% slough removed with day 4 showing 21.58%. The same trend was exhibited by making a large increase by day 8 at 78.88% and then having a full 100% slough removal by day 11. Appendix 3 shows the raw data for slough removal

All treatments groups were compared as shown in Figure 26. Those wounds treated with Revity showed a substantially higher slough removal percentage during both initial assessment days. On day 0, Revity wounds reached 90.70%, while both Saline Gauze and Untreated Control reached below 10%.

Figure 26: Percentage of Slough Removed after treatment application



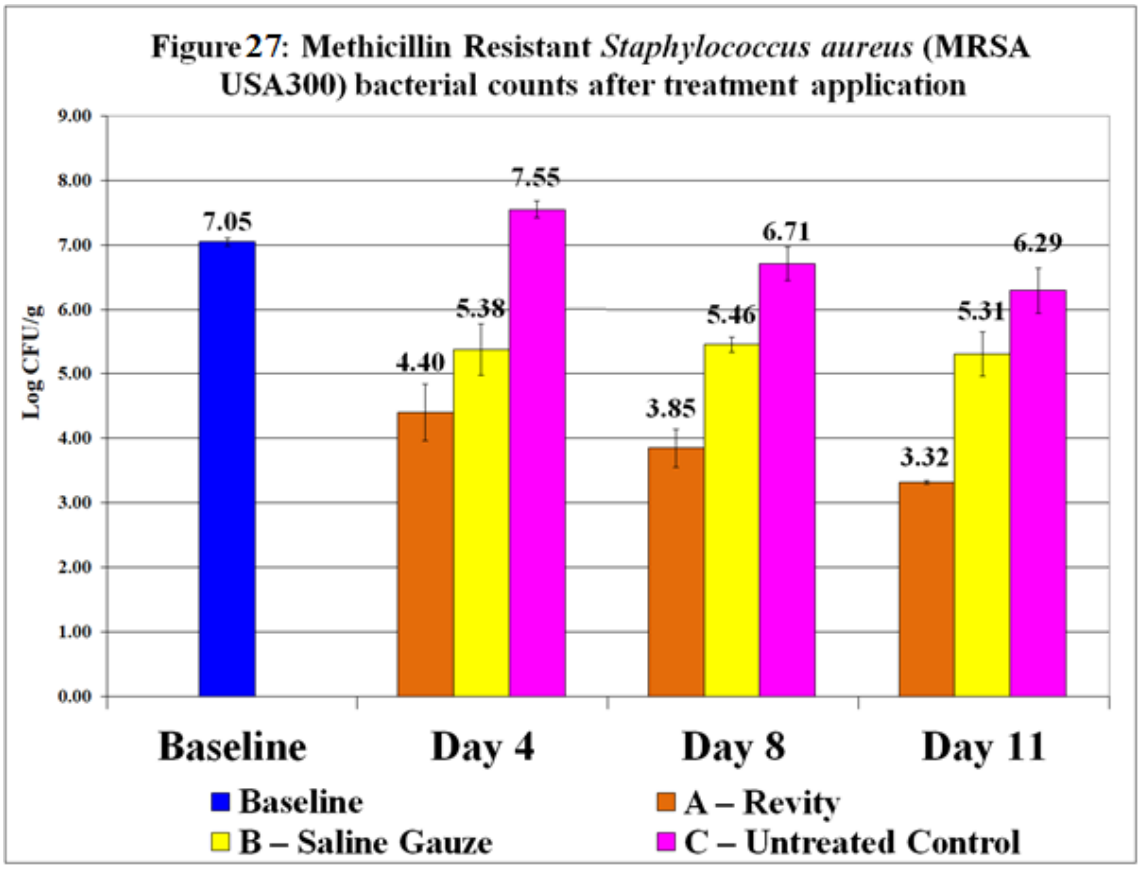
Approximately the same difference was shown on day 4 with both Saline Gauze and Untreated Control reaching 20.99 and 21.58%, respectively, while Revity wounds had far more slough removed at 96.67%. On day 8, there was a large difference in percentages between Revity and Untreated

Control, with Revity treated wounds reaching a complete slough removal (100%). Those wounds treated with Saline Gauze (87.96%), were slightly higher than those left untreated (78.88%). On day 11, all wounds from every group reached 100% slough removal as shown in Figure 26.

Microbiology

After counting the colonies, the data was tabulated and the Log of colony forming units/ml (Log CFU/g) was determined. The mean of the Log (CFU/g) were calculated for each time and treatment. Appendix 4 contains the raw data.

On day 0 (three days after wounding and infection), baseline wounds were recovered for an initial microbial count. Baseline wounds showed a bacterial count of 7.05±0.06 Log CFU/g. On day 4, Untreated Control wounds showed the highest MRSA counts at 7.55±0.14 Log CFU/g, which was the highest bacterial count during the entire study. Those wounds treated with Revity exhibited a bacterial count of 4.40±0.44 Log CFU/g (99.77 and 99.93% bacterial reductions when



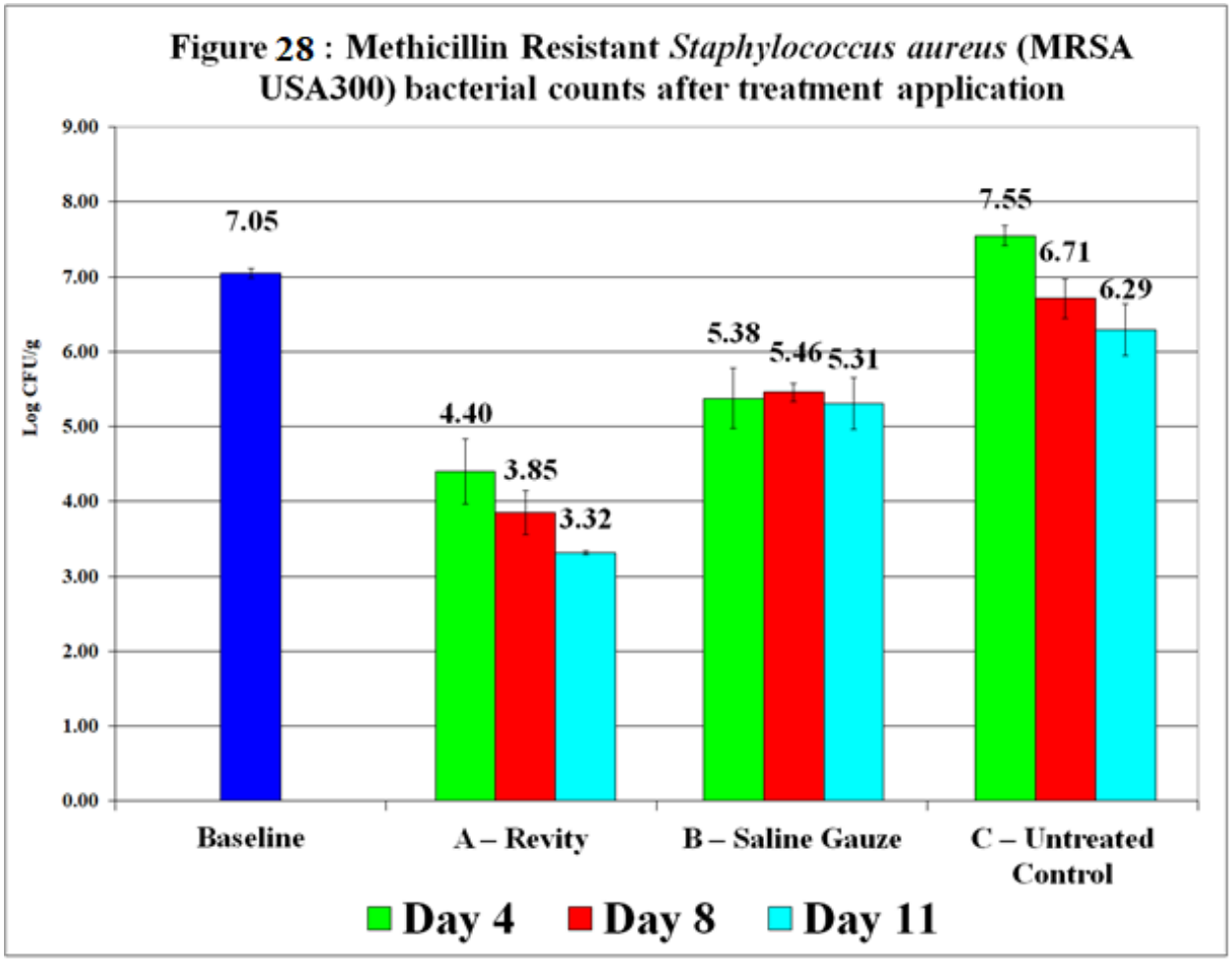
compared baseline wounds and Untreated Control, respectively). Revity treated wounds had the lowest microbial counts for this timepoint when compared against other treatment groups. Those wounds treated with Saline Gauze showed almost approximately 1 Log CFU/g bacterial count more than Revity treated wounds (5.38±0.40 Log CFU/g). These wounds had a bacterial reduction of 99.33% when compared against Untreated Control.

On day 8, those wounds left untreated reached the highest bacterial count among all groups on this timepoint at 6.71±0.14 Log CFU/g. Those wounds treated with Revity exhibited the lowest MRSA count on day 8 (3.85±0.30 Log CFU/g), having a bacterial reduction of 99.94% when compared against baseline wounds. Revity wounds were substantially lower than both Saline Gauze and Untreated Control (97.52 and 99.86% bacterial reductions, respectively). Saline Gauze wounds had a bacterial count of 5.46±0.12 Log CFU/g (97.44 and 94.43% bacterial reductions when compared against baseline wounds and Untreated Control, respectively).

Untreated wounds on day 11 showed the highest MRSA presence when compared against the other treatment groups at 6.29±0.35 Log CFU/g, as shown in Figure 27. The same trend was observed as the previous timepoints with Revity treated wounds having the lowest bacterial counts (3.32±0.02 Log CFU/g) when compared against all other groups. Revity wounds were substantially lower than baseline wounds (99.98% baseline reduction). When compared against Saline Gauze and Untreated Control, there were bacterial reductions of 98.97 and 99.89%, respectively. Those wounds treated with Saline Gauze (5.31±0.35 Log CFU/g) were also lower than both baseline wounds and Untreated Control (98.19 and 89.61%, respectively). However, the difference between Revity treated wounds was substantially larger than Saline Gauze treated wounds results.

Each assessment day MRSA counts within their own respective treatment group was showed in Figure 28. Those wounds treated with Revity exhibited a decreasing trend from day 4 to day 11 with a bacterial difference of 1.08±0.41 Log CFU/g which yields a bacterial reduction of 91.74%. A

similar trend resulted with wounds left untreated. Wounds left untreated on Day 4 exhibited the highest value and then continued to decrease until day 11. There were bacterial reductions of 85.54 and 94.51%, when comparing day 4 against both days 8 and 11, respectively. Saline Gauze treated wounds did not show the same reducing trend as Revity and Untreated Control. The bacterial counts values remained within the range of 5.46 and 5.31 Log CFU/g during the three time points. Saline Gauze exhibited values that were both higher than Revity while simultaneously exhibit lower bacterial counts than Untreated Control during each assessment day. On days 8 and 11 wounds treated with Revity showed a bacterial reduction highest than 97% in both days compared with wounds treated with Saline Gauze.



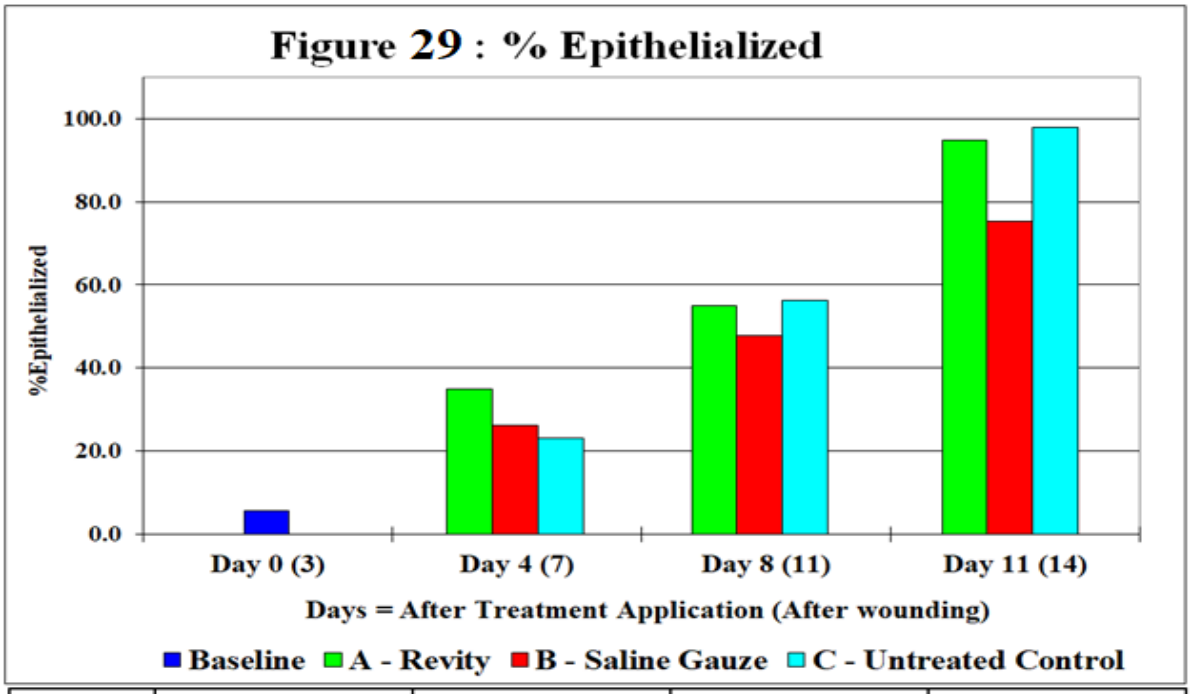
Histology Results

The histological analysis was performed blindly without knowing the treatment for each group. Wounds assessed in each time point were analyzed and the mean values presented below. Each histological parameter was represented for days after treatment (days 0, 4, 8 and 11). The values inside parenthesis represent days after wounding and infected (days 3, 7, 11 and 14).

Percentage of Re-Epithelialization

The percent of re-epithelialization represents the percent of the wound area covered by newly formed epidermis with one or more layers of keratinocytes, which is a good index for the speed of keratinocyte migration and the first step of the re-epithelialization.

Baseline wounds were recovered three days after wounding and infection, these wound only showed a 5.7% of re-epithelialization as shown in Figure 29. On day 4 (7 days after wounding), wounds treated with Revity exhibited the highest amount of re-epithelialization (34.8%) when compared against the other treatment groups. Both Saline Gauze and Untreated Control had 26.2 and



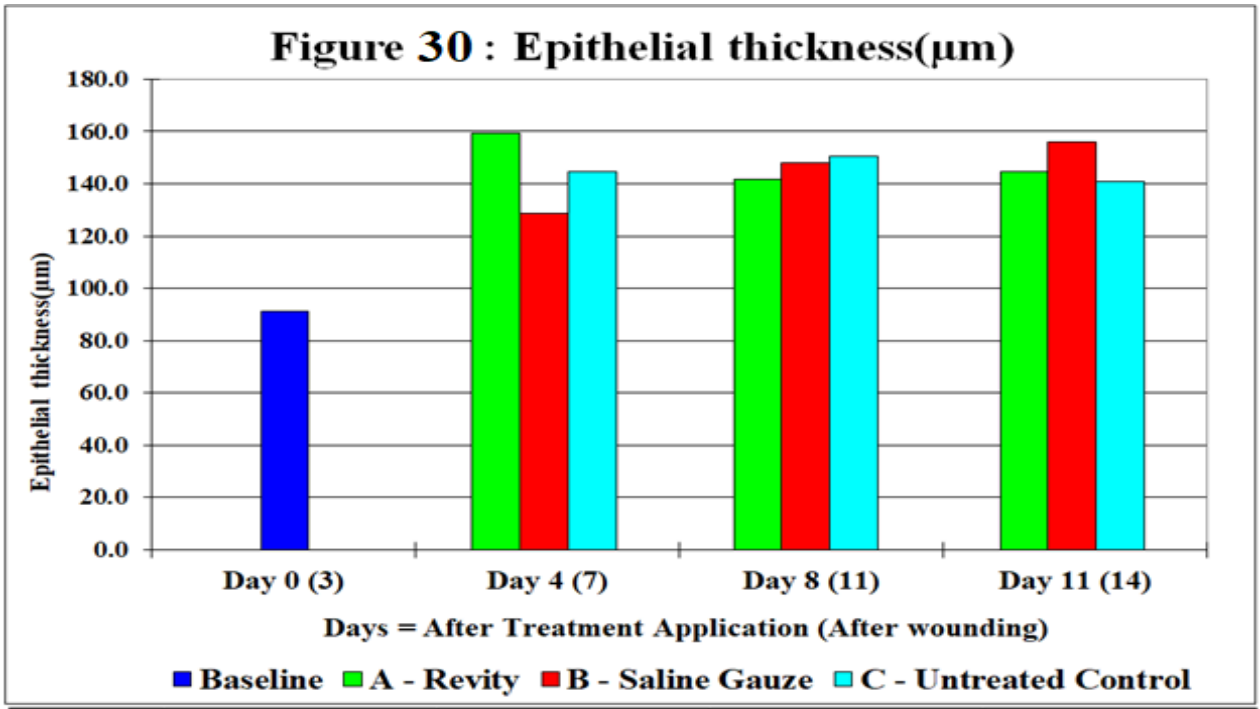
%Epithelialized	Baseline	A - Revity	B - Saline Gauze	C - Untreated Control
Day 0 (3)	5.7	0.0	0.0	0.0
Day 4 (7)	0.0	34.8	26.2	23.2
Day 8 (11)	0.0	54.8	47.9	56.4
Day 11 (14)	0.0	94.8	75.3	97.9

23.2%, respectively. By day 8, those wounds left untreated (56.4%) reached similar values to Revity treated wounds (54.8%). While Saline Gauze showed a lower re-epithelialization (47.9%). By day 11, both Revity and Untreated Control had the highest re-epithelialization with 94.8 and 97.9%, respectively. Those wounds treated with Saline Gauze showed the lowest value at 75.3%.

Epithelial Thickness:

The epithelial thickness was a measure of an average thickness of five points of newly formed epithelium. Epithelial thickness reflects the process of keratinocyte proliferation, differentiation, and epidermal maturation.

Baseline wounds exhibited epithelial thickness measurements of 91.2 µm on day 0. On day 4, those wounds treated with Revity exhibited the highest epithelial thickness measurement (159.2 µm) in the entire study. Those wounds treated with Saline Gauze (128.8 µm) and Untreated Control (144.8 µm) had higher measurements than baseline wounds. On day 8, all wounds had slightly similar



Epithelial thickness(um)	Baseline	A - Revity	B - Saline Gauze	C - Untreated Control
Day 0 (3)	91.2	0.0	0.0	0.0
Day 4 (7)	0.0	159.2	128.8	144.8
Day 8 (11)	0.0	141.6	148.0	150.4
Day 11 (14)	0.0	144.8	156.0	140.8

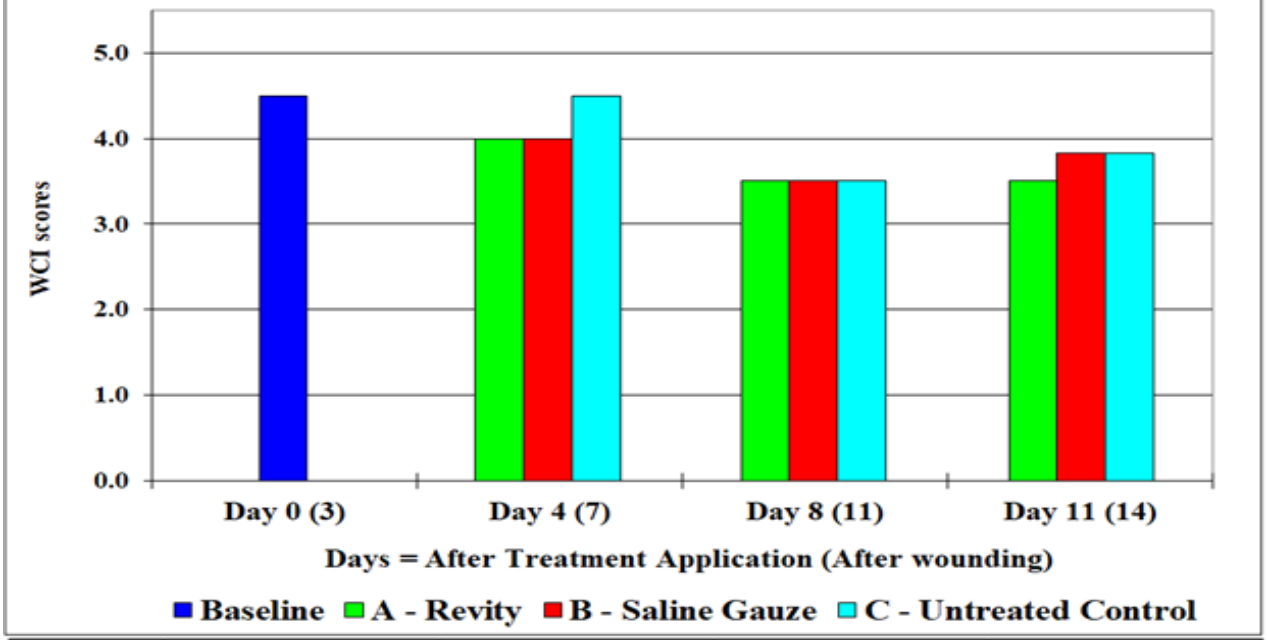
measurements, as shown in Figure 30. Revity treated wounds (141.6 µm) reducing when compared against its own respective epithelial thickness measurement from the previous time point. On day 11, those wounds treated with Saline Gauze had the highest measurement (156.0 µm) when compared against the other groups. Revity treated wounds (144.8 µm) were slightly higher than Untreated control (140.8µm).

White Cell Infiltration:

White cell infiltration (WCI) is used to access the inflammation reaction that could be a normal process of wound repair or due to microbial infection or the tissue reaction to foreign materials in the wound.

On day 0, baseline wounds exhibited a white cell infiltration score of 4.5 (Figure 31). On day 4, both Revity and Saline Gauze treated wounds had the same WCI scores at 4.0, while those wounds left untreated reached the highest score (4.5) among groups on this time point. On day 8, all wounds

Figure 31 : White cell infiltration



White cell infiltration	Baseline	A - Revity	B - Saline Gauze	C - Untreated Control
Day 0 (3)	4.5	0.0	0.0	0.0
Day 4 (7)	0.0	4.0	4.0	4.5
Day 8 (11)	0.0	3.5	3.5	3.5
Day 11 (14)	0.0	3.5	3.8	3.8

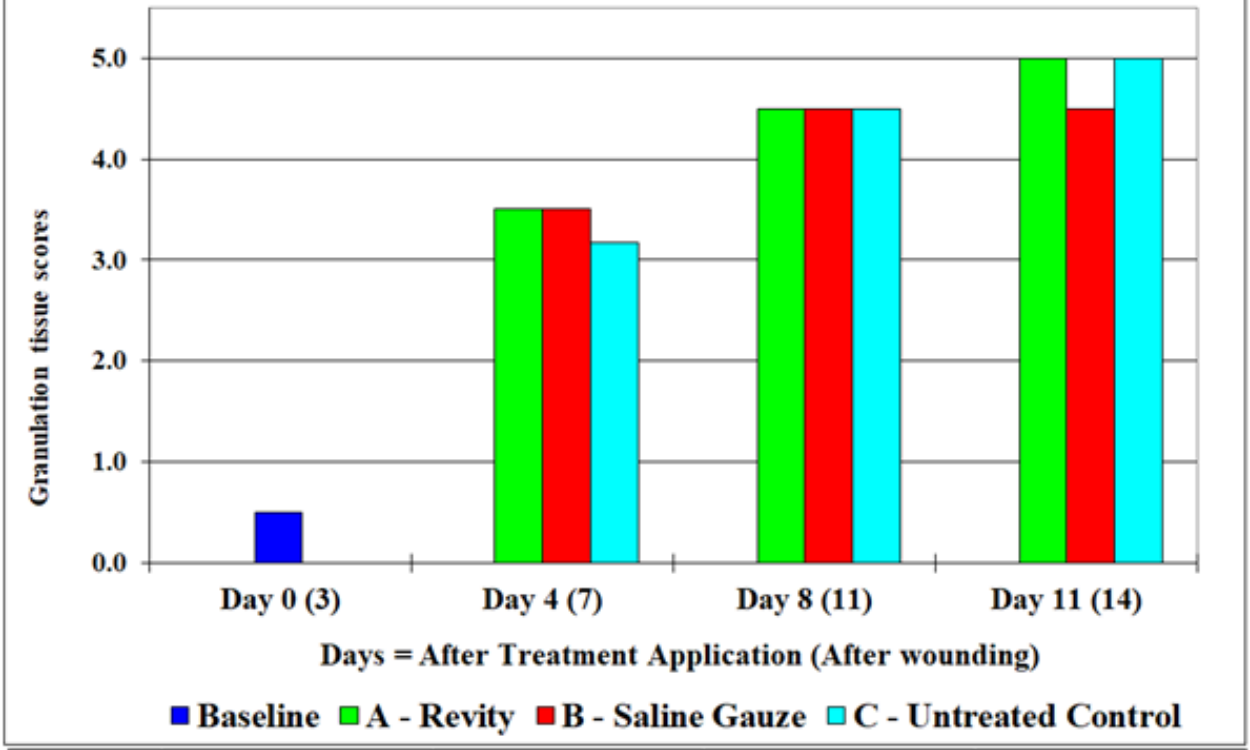
showed WCI scores of 3.5. On day 11, those wounds treated with Revity had the lowest score (3.5) when compared against Saline Gauze and Untreated Control (both at 3.8).

Granulation Tissue Formation

The dermal reconstitution begins in about 3 to 4 days of injury with the hallmark of granulation tissue formation, which include new blood vessel formation (angiogenesis), and the accumulation of fibroblasts and collagen extracellular matrices. The granulation tissue formation measures the percent of wound bed filled with newly formed granulation tissue.

Baseline wounds recovered on day 0 had a score of 0.5 for granulation tissue formation. On day 4, both Revity and Saline Gauze exhibited scores of 3.5, having a slightly higher score than those

Figure 32 : Granulation tissue formation



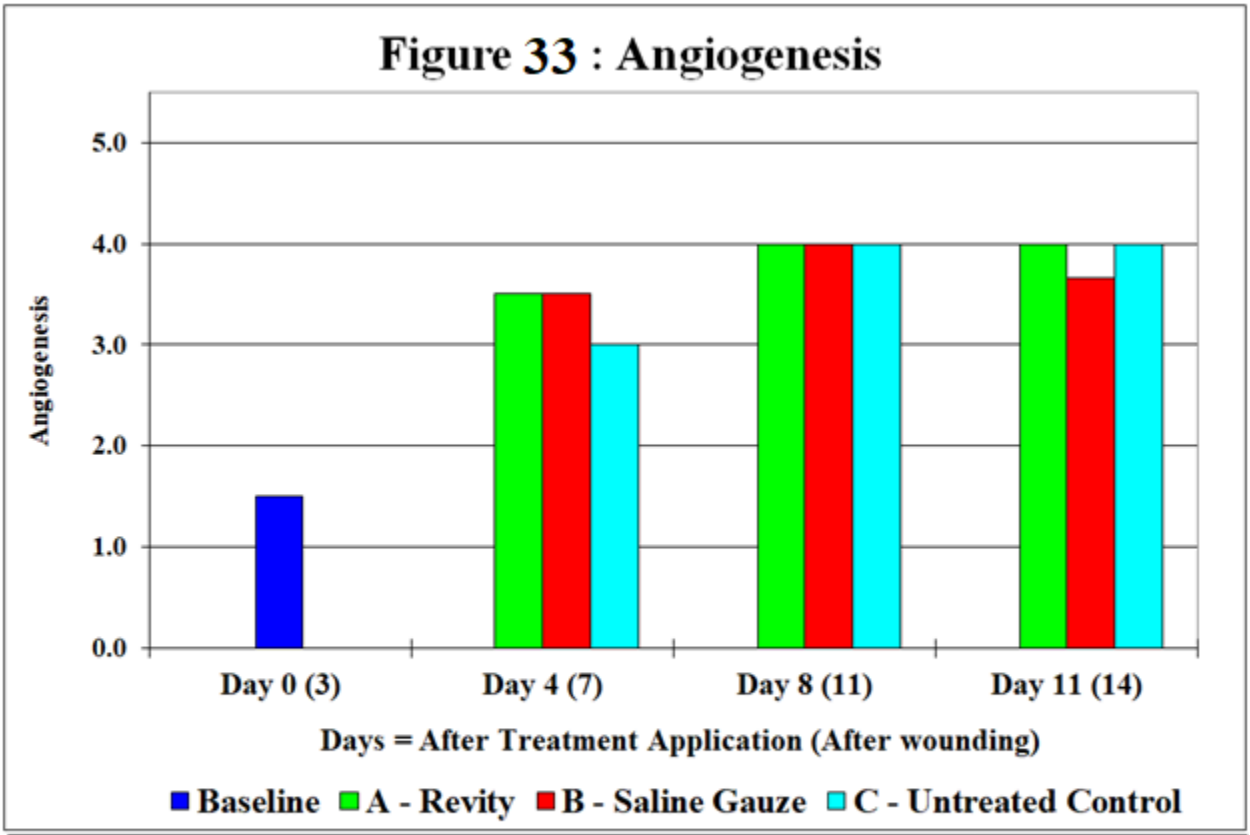
Granulation tissue formation	Baseline	A - Revity	B - Saline Gauze	C - Untreated Control
Day 0 (3)	0.5	0.0	0.0	0.0
Day 4 (7)	0.0	3.5	3.5	3.2
Day 8 (11)	0.0	4.5	4.5	4.5
Day 11 (14)	0.0	5.0	4.5	5.0

left untreated (3.2). Figure 32 shows all wounds having the same granulation tissue formation score on day 8 (4.5). On day 11, those wounds treated with Revity and Untreated Control reached the highest score (5.0), while those wounds being treated with Saline Gauze (4.5) did not change its results from the previous timepoint.

Angiogenesis:

Angiogenesis measures the degree of new microvascular blood vessel formation, which is characterized by newly formed capillary blood vessels with proliferating endothelial cells sprouting from adjacent existing blood vessels.

Figure 33 : Angiogenesis



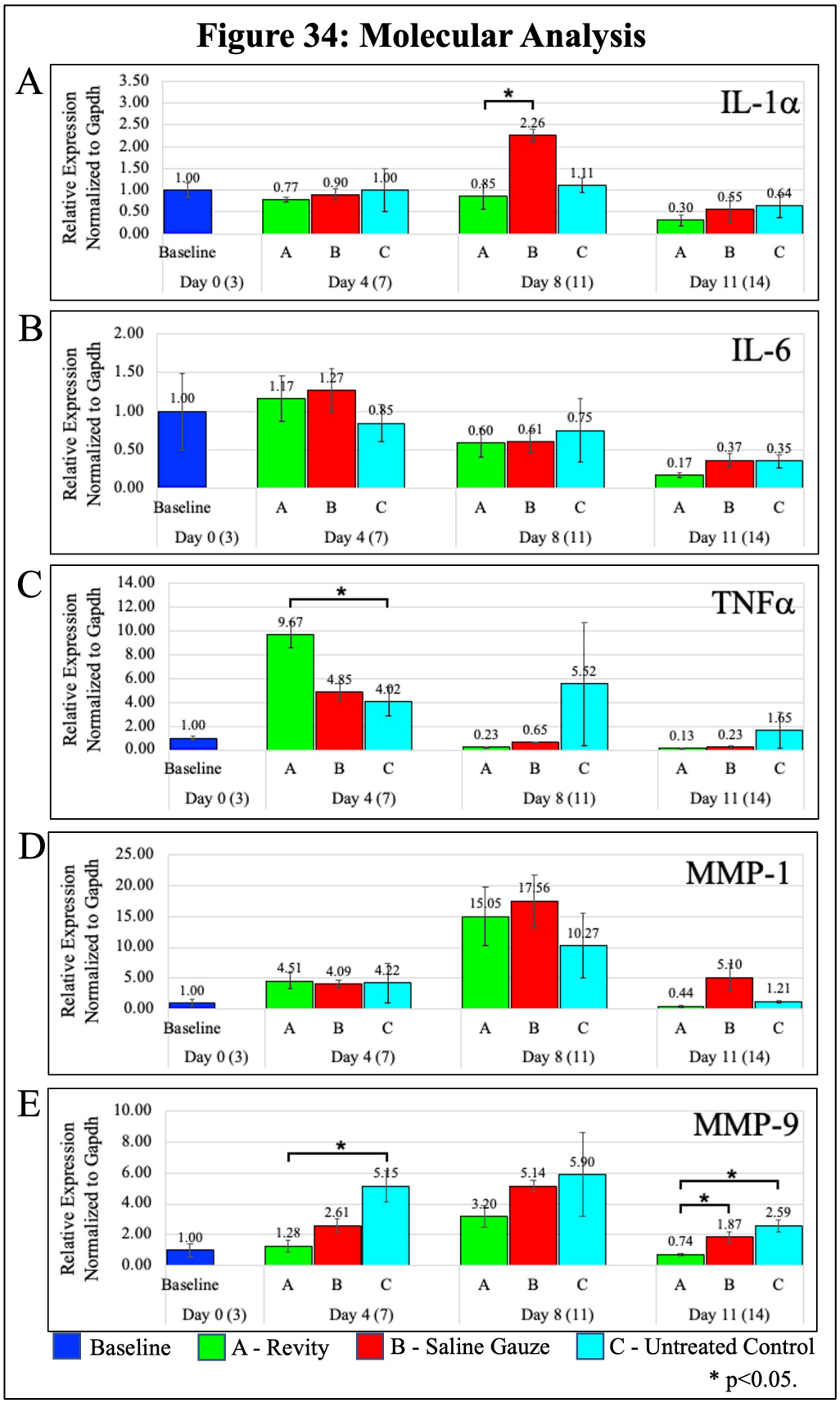
Angiogenesis	Baseline	A - Revity	B - Saline Gauze	C - Untreated Control
Day 0 (3)	1.5	0.0	0.0	0.0
Day 4 (7)	0.0	3.5	3.5	3.0
Day 8 (11)	0.0	4.0	4.0	4.0
Day 11 (14)	0.0	4.0	3.7	4.0

On day 0, baseline wounds showed an angiogenesis score of 1.5 (Figure 33). On day 4, those left untreated had the lowest angiogenesis score at 3.0, while both Revity and Saline Gauze exhibited similar scores (both at 3.5). On day 8, all wounds reached the same angiogenesis score (4.0). On day 11, both Revity and Untreated Control showed similar scores at 4.0, while those wounds treated with Saline Gauze had a slightly lower score (3.7).

Molecular Assessment

Real-time PCR was carried out to determine changes in inflammatory (IL-1α, IL-6, TNFα) and matrix remodeling (MMP-1, MMP-9) marker expression upon Revity treatment of the wounds. By day 8 after treatment (11 days after wounding), there was a 62% reduction in IL-1α expression level in Revity versus Saline Gauze-treated samples (Figure 34A), which was statistically significant ($p<0.05$). As expected, increased TNFα levels were observed in all the samples 7 days after wounding (day 4 after treatment) compared with baseline (Figure 34C), and the relative TNFα levels were significantly higher in Revity treated versus untreated samples ($p<0.05$) (Figure 34C). As wound healing progressed and MRSA counts were reduced in Revity and Saline Gauze-treated wounds, the expression of TNFα became much reduced (Day 8, Day 11, Figure 34C). Upon wounding, expression of MMP-1 and MMP-9 was increased in all the samples with or without Revity treatment, with untreated samples showing the most robust increase (Figure 34D, E). MMP-9 expression levels in the Revity-treated samples were closest to baseline and were significantly lower than Saline Gauze treated or untreated samples (Figure 34E). Raw data can be found in Appendix 5.

Figure 34: Molecular Analysis



CONCLUSIONS

Wounds treated with Revity had a higher percentage of slough removal and MRSA reduction. Revity treated wounds had a desirable effect on slough removal the day of treatment (day 0) and 4 days after this single application the count reached more than 99 % of bacterial reduction compared with the baseline and untreated wounds. The effects were noticeable when compared against the other groups. Ultimately, Revity was able to reduce the MRSA microbial counts by half compared with Untreated Control on every assessment day. This could have occurred due to the substantial amounts of slough removed by Revity since day 0. Additionally in the histology analysis, those wounds treated with Revity showed high values of re-epithelialization on day 4. Consistently, while Revity treated samples showed an initial very robust increase in TNFα expression levels, TNFα expression was rapidly reduced as wound healing progressed. Revity did not inhibit the wound healing process since Revity treated wounds almost reached full re-epithelialization by day 11. Additional studies with more animals would be needed to substantiate these claims and acquire statistical data.

APPENDIX 1. Slough and Coagulum.

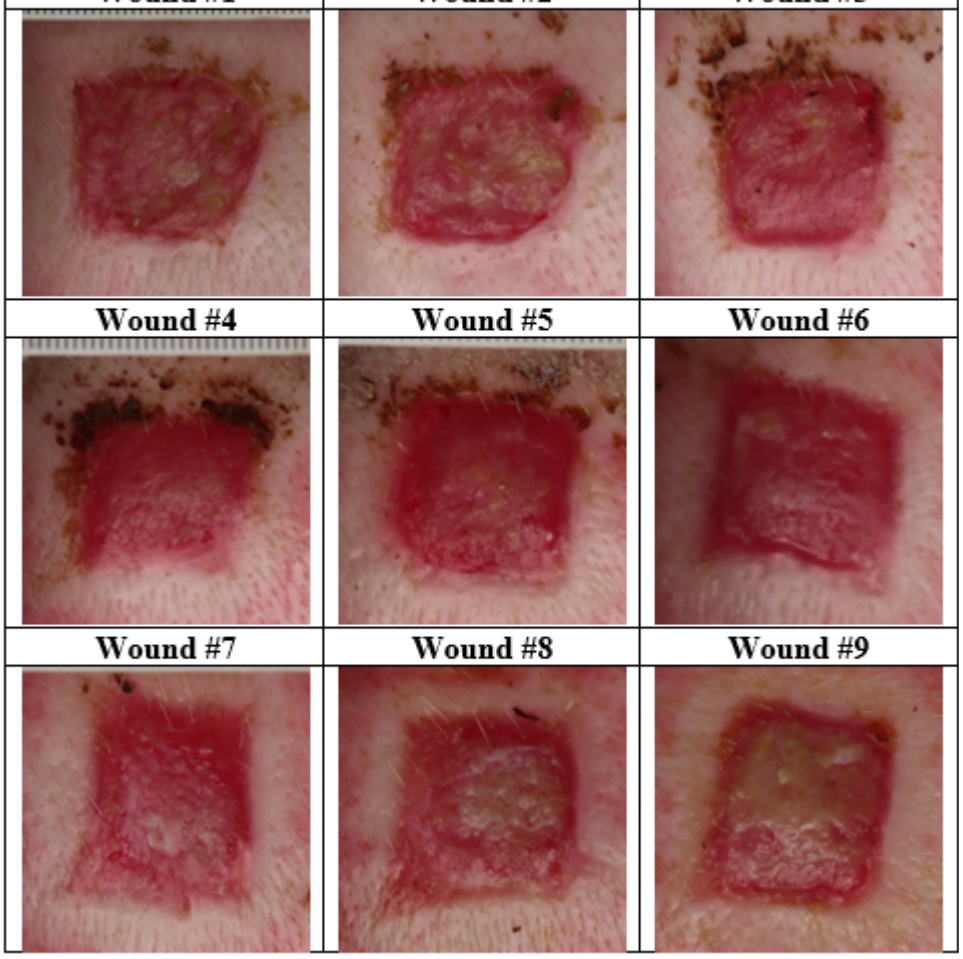
Table 1: Slough Observations

EPIEN Medical, Inc.			**P21 226/27**			
Slough - degree of moist devitalized tissue*						
* Score: 1 = absent, 2 = mild, 3 = moderate, 4 = marked, 5 = exuberant						
Day 0				**Day 4**	**Day 8**	**Day 11**
Wounds	**Treatment (Rx)**	**Slough (before wipe)**	**Slough (after wipe)**	**Slough**	**Slough**	**Slough**
1	Baseline	5	N/A	N/A	N/A	N/A
2	Baseline	5	N/A	N/A	N/A	N/A
3	Baseline	5	N/A	N/A	N/A	N/A
1	Revity	5	2	2	1	N/A
2	Revity	5	2	2	1	N/A
3	Revity	5	2	1	1	N/A
4	Revity	5	2	1	1	1
5	Revity	5	2	1	1	1
6	Revity	5	2	1	1	1
7	Revity	5	2	1	N/A	N/A
8	Revity	5	2	1	N/A	N/A
9	Revity	5	2	2	N/A	N/A
1	Saline Irrigation	5	4	5	2	N/A
2	Saline Irrigation	5	3	5	2	N/A
3	Saline Irrigation	5	4	5	2	N/A
4	Saline Irrigation	5	4	3	1	1
5	Saline Irrigation	5	4	4	2	1
6	Saline Irrigation	5	4	5	3	1
7	Saline Irrigation	5	4	5	N/A	N/A
8	Saline Irrigation	5	4	5	N/A	N/A
9	Saline Irrigation	5	4	5	N/A	N/A
1	Untreated Control	5	4	5	N/A	N/A
2	Untreated Control	5	4	5	N/A	N/A
3	Untreated Control	5	4	2	N/A	N/A
4	Untreated Control	5	4	3	2	N/A
5	Untreated Control	5	4	5	4	1
6	Untreated Control	5	3	5	1	1
7	Untreated Control	5	3	5	2	1
8	Untreated Control	5	3	5	2	N/A
9	Untreated Control	5	4	5	3	N/A

Table 2: Coagulum Observations

DAY 4													
Treatment	Wounds	Area Total				Area Total (cm2)	Treatment		Coagulum Area				Area of Coagulum (cm2)	Percentage of Coagulum
A	1	328840	7.364	0	255	57.55	A	1	45737	7.616	0	255	8.00	13.91
A	2	376168	23.136	0	255	65.83	A	2	125008	7.616	0	255	21.88	33.23
A	3	334987	0.039	0	255	58.63	A	1	46533	0.039	0	255	8.14	13.89
A	4	441296	8.087	0	255	77.23	A	4	12612	7.616	0	255	2.21	2.86
A	5	494298	2.548	0	255	86.51	A	5	9625	7.616	0	255	1.68	1.95
A	6	418662	4.285	0	255	73.27	A	6	73233	7.616	0	255	12.82	17.49
A	7	504628	6.392	0	255	88.32	A	7	76689	7.616	0	255	13.42	15.20
A	8	708492	6.927	0	255	124.00	A	8	504325	7.616	0	255	88.26	71.18
A	9	593196	8.943	0	255	103.82	A	9	478196	7.616	0	255	83.69	80.61
														Average of percentage **27.81**

Figure A: Revity (Treatment A)



APPENDIX 2. Erythema and bleeding

Figure B: Erythema Comparison

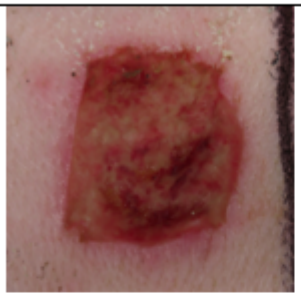
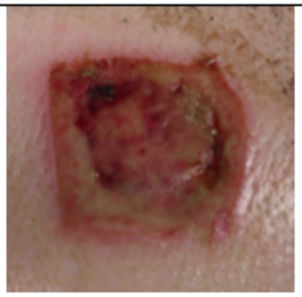
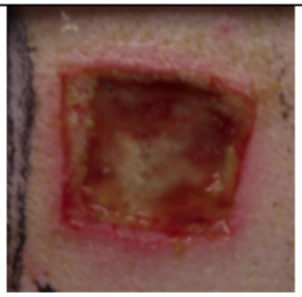
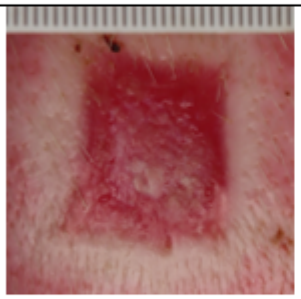
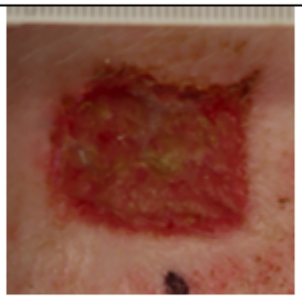
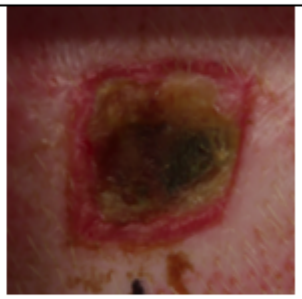
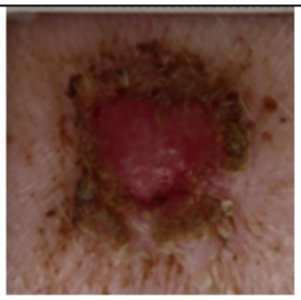
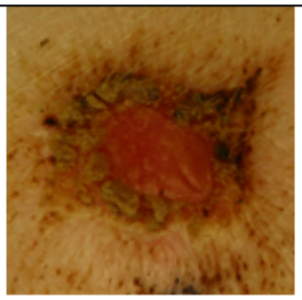
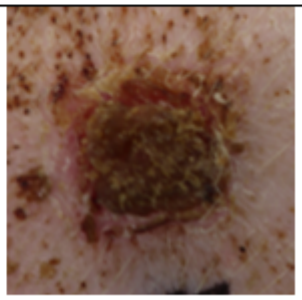
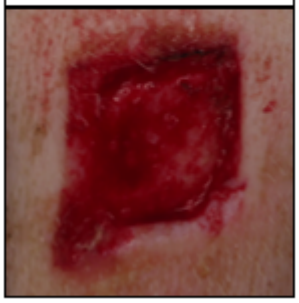
	Revity	Saline Irrigation	Untreated Control
Day 0			
Day 4			
Day 8			

Figure C



APPENDIX 3. Raw data for slough percentage of removal

DAY 0

Treatment	Wounds	Before Wiping				Area Total (cm2)	Treatment		After Wiping				Area of Slough (cm2)	Percentage Removed
A	1	1249789	0.109	0	255	218.73	A	1	357283	7.616	0	255	62.53	71.41
A	2	1068535	0.026	0	255	187.01	A	2	197266	7.616	0	255	34.52	81.54
A	3	1505811	0.081	0	255	263.54	A	3	103370	7.616	0	255	18.09	93.14
A	4	1385192	0.04	0	255	242.43	A	4	27096	7.616	0	255	4.74	98.04
A	5	1271636	0.07	0	255	222.55	A	5	42551	7.616	0	255	7.45	96.65
A	6	950560	0.051	0	255	166.36	A	6	25487	7.616	0	255	4.46	97.32
A	7	815377	0.825	0	255	142.70	A	7	92210	7.616	0	255	16.14	88.69
A	8	886833	2.649	0	255	155.21	A	8	28003	7.616	0	255	4.90	96.84
A	9	602944	0	0	0	105.52	A	9	44279	7.616	0	255	7.75	92.66
														Average of percentage
														90.70

DAY 0

Treatment	Wounds	Before Wiping				Area Total (cm2)	Treatment		After Wiping				Area of Slough (cm2)	Percentage Removed
B	1	1667689	0.751	0	255	291.87	B	1	1516807	7.616	0	255	265.46	9.05
B	2	1475199	0.003	0	255	258.18	B	2	1385790	7.616	0	255	242.53	6.06
B	3	1677914	0.004	0	255	293.66	B	3	1561679	7.616	0	255	273.31	6.93
B	4	1595797	0.113	0	255	279.29	B	4	1509849	7.616	0	255	264.24	5.39
B	5	1172993	0.228	0	255	205.29	B	5	1076840	7.616	0	255	188.46	8.20
B	6	1319957	0.02	0	255	231.01	B	6	1267036	7.616	0	255	221.75	4.01
B	7	974116	2.792	0	255	170.48	B	7	931140	7.616	0	255	162.96	4.41
B	8	955031	20.113	0	255	167.14	B	8	917616	7.616	0	255	160.60	3.92
B	9	1077455	4.213	0	255	188.57	B	9	916565	0.951	0	255	160.41	14.93
														Average of percentage
														6.99

DAY 0

Treatment	Wounds	Before Wiping				Area Total (cm2)	Treatment		After Wiping				Area of Slough (cm2)	Percentage Removed
C	**1**	765868	0.256	0	255	134.04	C	1	641054	7.616	0	255	112.19	16.30
C	**2**	738219	3.313	0	255	129.20	C	2	675015	7.616	0	255	118.14	8.56
C	**3**	853698	0.002	0	255	149.41	C	3	797849	7.616	0	255	139.63	6.54
C	4	755743	0.001	0	255	132.27	C	4	713443	7.616	0	255	124.86	5.60
C	**5**	872770	0	0	0	152.75	C	5	789760	7.616	0	255	138.22	9.51
C	6	1319957	0.02	0	255	231.01	C	6	1294470	7.616	0	255	226.55	1.93
C	**7**	863575	0.016	0	255	151.14	C	7	791095	7.616	0	255	138.45	8.39
C	**8**	656257	2.178	0	255	114.85	C	8	616546	7.616	0	255	107.90	6.05
C	**9**	581845	1.718	0	255	101.83	C	9	463114	7.616	0	255	81.05	20.41
														Average of percentage
														9.25

DAY 4

Treatment	Wounds	Before Wiping				Area Total (cm2)	Treatment		After Wiping				Area of Slough (cm2)	Percentage Removed
A	1	346341	0.11	0	255	60.61	A	1	12635	7.616	0	255	2.21	96.35
A	2	361028	7.042	0	255	63.18	A	2	13199	7.616	0	255	2.31	96.34
A	3	334987	0.039	0	255	58.63	A	1	7386	0.039	0	255	1.29	97.80
A	4	441296	8.087	0	255	77.23	A	4	6616	7.616	0	255	1.16	98.50
A	5	494298	2.548	0	255	86.51	A	5	3626	7.616	0	255	0.63	99.27
A	6	418662	4.285	0	255	73.27	A	6	5713	7.616	0	255	1.00	98.64
A	7	504628	6.392	0	255	88.32	A	7	11403	7.616	0	255	2.00	97.74
A	8	708492	6.927	0	255	124.00	A	8	48186	7.616	0	255	8.43	93.20
A	9	593196	8.943	0	255	103.82	A	9	46057	7.616	0	255	8.06	92.24
														Average of percentage
														96.67

DAY 4

Treatment	Wounds	Before Wiping				Area Total (cm2)	Treatment		After Wiping				Area of Slough (cm2)	Percentage Removed
B	1	569820	0.058	0	255	99.73	B	1	465530	7.616	0	255	81.47	18.30
B	2	628738	0.055	0	255	110.04	B	2	367170	7.616	0	255	64.26	41.60
B	3	634396	0.214	0	255	111.03	B	3	564649	7.616	0	255	98.82	10.99
B	4	609561	0.009	0	255	106.68	B	4	406786	7.616	0	255	71.19	33.27
B	5	463169	0.003	0	255	81.06	B	5	361988	7.616	0	255	63.35	21.85
B	6	432191	0.013	0	255	75.64	B	6	378357	7.616	0	255	66.22	12.46
B	7	329584	0	0	0	57.68	B	7	263899	7.616	0	255	46.19	19.93
B	8	345014	0.001	0	255	60.38	B	8	289487	7.616	0	255	50.66	16.09
B	9	545537	0.001	0	255	95.48	B	9	466677	0.951	0	255	81.67	14.46
														Average of percentage
														20.99

DAY 4

Treatment	Wounds	Before Wiping				Area Total (cm2)	Treatment		After Wiping				Area of Slough (cm2)	Percentage Removed
C	1	852612	0.01	0	255	149.22	C	1	667245	7.616	0	255	116.78	21.74
C	2	712858	0.005	0	255	124.76	C	2	577236	7.616	0	255	101.02	19.03
C	3	513609	0.009	0	255	89.89	C	3	426772	7.616	0	255	74.69	16.91
C	4	457937	0.006	0	255	80.15	C	4	325847	7.616	0	255	57.03	28.84
C	5	818794	0.015	0	255	143.30	C	5	554597	7.616	0	255	97.06	32.27
C	6	823753	0.006	0	255	144.17	C	6	718212	7.616	0	255	125.70	12.81
C	7	730132	0.01	0	255	127.78	C	7	538034	7.616	0	255	94.16	26.31
C	8	501351	0.001	0	255	87.74	C	8	405552	7.616	0	255	70.98	19.11
C	9	429590	5.94E-04	0	255	75.18	C	9	355712	7.616	0	255	62.25	17.20
														Average of percentage
														21.58

DAY 8

Treatment	Wounds	Before Wiping				Area Total (cm2)	Treatment		After Wiping				Area of Slough (cm2)	Percentage Removed
A	1	532268	0.003	0	255	93.15	A	1	532268	0.003	0	255	93.15	100.00
A	2	446852	0.001	0	255	78.21	A	2	446852	0.001	0	255	78.21	100.00
A	3	449672	0.059	0	255	78.70	A	3	449672	0.059	0	255	78.70	100.00
A	4	549111	0.051	0	255	96.10	A	4	549111	0.051	0	255	96.10	100.00
A	5	536969	0.022	0	255	93.98	A	5	536969	0.022	0	255	93.98	100.00
A	6	397574	0.838	0	255	69.58	A	6	397574	0.838	0	255	69.58	100.00
														Average of percentage
														100.00

DAY 8

Treatment	Wounds	Before Wiping				Area Total (cm2)	Treatment		After Wiping				Area of Slough (cm2)	Percentage Removed
B	1	593811	0.079	0	255	103.92	B	1	53175	7.616	0	255	9.31	91.05
B	2	582642	0.019	0	255	101.97	B	2	83705	7.616	0	255	14.65	85.63
B	3	684100	0.103	0	255	119.73	B	3	138802	7.616	0	255	24.29	79.71
B	4	585099	4.845	0	255	102.40	B	4	15131	0.522	0	255	2.65	97.41
B	5	345806	16.705	0	255	60.52	B	5	32252	7.616	0	255	5.64	90.67
B	6	435342	20.205	0	255	76.19	B	6	72837	7.616	0	255	12.75	83.27
														Average of percentage
														87.96

DAY 8

Treatment	Wounds	Before Wiping				Area Total (cm2)	Treatment		After Wiping				Area of Slough (cm2)	Percentage Removed
C	4	284845	36.995	0	255	49.85	C	4	46691	7.616	0	255	8.17	83.61
C	5	559103	0.082	0	255	97.85	C	5	102878	7.616	0	255	18.01	81.60
C	6	610746	0.067	0	255	106.89	C	6	55606	0.055	0	255	9.73	90.90
C	7	829404	0.018	0	255	145.16	C	7	204104	7.616	0	255	35.72	75.39
C	8	736145	0.001	0	255	128.84	C	8	243629	7.616	0	255	42.64	66.90
C	9	477736	0.015	0	255	83.61	C	9	119869	7.616	0	255	20.98	74.91
														Average of percentage
														78.88

DAY 11

Treatment	Wounds	Before Wiping				Area Total (cm2)	Treatment		After Wiping				Area of Slough (cm2)	Percentage Removed
A	4	382025	0.007	0	255	66.86	A	4	382025	7.616	0	255	66.86	100.00
A	5	398952	0.056	0	255	69.82	A	5	398952	7.616	0	255	69.82	100.00
A	6	259953	0.023	0	255	45.50	A	6	259953	7.616	0	255	45.50	100.00
														Average of percentage
														100.00

DAY 11

Treatment	Wounds	Before Wiping				Area Total (cm2)	Treatment		After Wiping				Area of Slough (cm2)	Percentage Removed
B	4	568793	0.015	0	255	99.55	B	4	568793	7.616	0	255	99.55	100.00
B	5	622974	0.021	0	255	109.03	B	5	622974	7.616	0	255	109.03	100.00
B	6	750671	0	0	0	131.38	B	6	750671	7.616	0	255	131.38	100.00
														Average of percentage
														100.00

DAY 11

Treatment	Wounds	Before Wiping				Area Total (cm2)	Treatment		After Wiping				Area of Slough (cm2)	Percentage Removed
C	4	196671	1.163	0	255	34.42	C	5	196671	7.616	0	255	34.42	100.00
C	8	492122	0.387	0	255	86.13	C	6	492122	7.616	0	255	86.13	100.00
C	9	461121	0.17	0	255	80.70	C	7	461121	7.616	0	255	80.70	100.00
														Average of percentage
														100.00

APPENDIX 4. Microbiology Raw Data

Determination of the Debridement Effects of Revity on Deep Dermal Wounds in a Porcine Model

Pig #1 P21-226/27

Inoculum

Strain	Dilution	Count	CFU/ml	Log CFU/ml
Methicillin Resistant Staphylococcus aureus (MRSA USA300)	-4	48	9.59E+06	6.98

Baseline 72 hours after wonding and infection

Treatment	Biopsy	Dilution	Count	CFU/ml	Log CFU/ml	
Baseline	1	-4	33	3.30E+07	7.52	
	2	-4	51	5.10E+07	7.71	
	3	-4	26	2.60E+07	7.41	STDV
			Mean	3.67E+07	7.55	0.15

Number of organism per g

Treatment	Biopsy	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
Baseline	1	33	5	10000	0.153	1.08E+07	7.03	
	2	51	5	10000	0.196	1.30E+07	7.11	
	3	26	5	10000	0.131	9.92E+06	7.00	STDV
					Mean	1.12E+07	7.05	0.06

Day 4

ORSAB Bacterial count in wounds recovered Day 4

Treatment	Biopsy	Dilution	Count	CFU/ml	Log CFU/ml	
A – Revity	7	-1	43	2.58E+04	4.41	
	8	-2	37	2.22E+05	5.35	
	9	-2	23	1.38E+05	5.14	STDV
			Mean	1.29E+05	4.97	0.49

Number of organism per g

Treatment	Biopsy	Number of	purpose	Dilution	Weight	CFU/g	Log CFU/g	
A – Revity	7	43	3	10	0.151	8.54E+03	3.93	
	8	37	3	100	0.179	6.20E+04	4.79	
	9	23	3	100	0.229	3.01E+04	4.48	STDV
					Mean	3.36E+04	4.40	0.44

ORSAB Bacterial count in wounds recovered Day 4

Treatment	Biopsy	Dilution	Count	CFU/ml	Log CFU/ml	
B – Saline Gauze	7	-2	44	2.66E+06	5.42	
	8	-3	21	1.26E+06	6.10	
	9	-3	36	2.16E+06	6.33	STDV
			Mean	2.03E+06	5.95	0.47

Number of organism per g

Treatment	Biopsy	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
B – Saline Gauze	7	44	3	100	0.159	8.30E+04	4.92	
	8	21	3	1000	0.178	3.54E+05	5.55	
	9	36	3	1000	0.237	4.56E+05	5.66	STDV
					Mean	2.98E+05	5.38	0.40

ORSAB Bacterial count in wounds recovered Day 4

Treatment	Biopsy	Dilution	Count	CFU/ml	Log CFU/ml	
C – Untreated Control	1	-5	24	1.44E+08	8.16	
	2	-5	29	1.74E+08	8.24	
	3	-4	178	1.07E+08	8.03	STDV
			Mean	1.42E+08	8.14	0.11

Number of organism per g

Treatment	Biopsy	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
C – Untreated Control	1	24	3	100000	0.196	3.67E+07	7.57	
	2	29	3	100000	0.183	4.75E+07	7.68	
	3	178	3	10000	0.209	2.56E+07	7.41	STDV
					Mean	3.66E+07	7.55	0.14

Day 8

ORSAB Bacterial count in wounds recovered Day 8

Treatment	Biopsy	Dilution	Count	CFU/ml	Log CFU/ml	
A – Revity	1	-1	81	3.24E+04	4.51	
	2	-1	126	5.04E+03	3.70	
	3	-1	34	1.36E+03	3.13	STDV
			Mean	1.29E+04	3.78	0.69

Number of organism per g

Treatment	Biopsy	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
A – Revity	1	81	2	10	0.181	8.95E+03	3.95	
	2	126	2	10	0.209	1.21E+04	4.08	
	3	34	2	10	0.207	3.29E+03	3.52	STDV
					Mean	8.10E+03	3.85	0.30

ORSAB Bacterial count in wounds recovered Day 8

Treatment	Biopsy	Dilution	Count	CFU/ml	Log CFU/ml	
B – Saline Gauze	1	-3	29	6.80E+03	3.83	
	2	-3	38	2.30E+04	4.36	
	3	-2	197	4.30E+04	4.63	STDV
			Mean	2.43E+04	4.27	0.41

Number of organism per g

Treatment	Biopsy	Colonies (N)	purpose	Factor (D)	Biopsy(g) X	CFU/g	Log CFU/g	
B – Saline Gauze	1	29	2	1000	0.190	3.05E+05	5.48	
	2	38	2	1000	0.211	3.60E+05	5.56	
	3	197	2	100	0.186	2.12E+05	5.33	STDV
					Mean	2.92E+05	5.46	0.12

ORSAB Bacterial count in wounds recovered Day 8

Treatment	Biopsy	Dilution	Count	CFU/ml	Log CFU/ml	
C – Untreated Control	5	-4	46	2.00E+06	6.30	
	6	-3	190	1.71E+06	6.23	
	7	-4	55	2.40E+06	6.38	STDV
			Mean	2.04E+06	6.30	0.08

Number of organism per g

Treatment	Biopsy	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
C – Untreated Control	5	46	3	10000	0.197	7.01E+06	6.85	
	6	190	3	1000	0.224	2.54E+06	6.41	
	7	55	3	10000	0.218	7.57E+06	6.88	STDV
					Mean	5.71E+06	6.71	0.26

Day 11

ORSAB Bacterial count in wounds recovered Day 11

Treatment	Biopsy	Dilution	Count	CFU/ml	Log CFU/ml	
A – Revity	4	0	197	7.88E+03	3.90	
	5	-1	28	1.12E+04	4.04	
	6	0	173	6.93E+03	3.84	STDV
			Mean	8.67E+03	3.93	0.10

Number of organism per g

Treatment	Biopsy	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
A – Revity	4	197	2	1	0.202	1.95E+03	3.29	
	5	28	2	10	0.259	2.16E+03	3.33	
	6	173	2	1	0.162	2.14E+03	3.33	STDV
					Mean	2.08E+03	3.32	0.02

ORSAB Bacterial count in wounds recovered Day 11

Treatment	Biopsy	Dilution	Count	CFU/ml	Log CFU/ml	
B – Saline Gauze	4	-3	44	1.76E+06	6.25	
	5	-2	78	3.12E+05	5.49	
	6	-3	20	8.00E+05	5.90	STDV
			Mean	9.57E+05	5.88	0.38

Number of organism per g

Treatment	Biopsy	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
B – Saline Gauze	4	44	2	1000	0.199	4.42E+05	5.65	
	5	78	2	100	0.173	9.02E+04	4.96	
	6	20	2	1000	0.192	2.08E+05	5.32	STDV
					Mean	2.47E+05	5.31	0.35

ORSAB Bacterial count in wounds recovered Day 11

Treatment	Biopsy	Dilution	Count	CFU/ml	Log CFU/ml	
C – Untreated Control	4	-3	128	7.68E+06	6.89	
	8	-4	31	1.86E+07	7.27	
	9	-3	63	3.78E+06	6.58	STDV
			Mean	1.00E+07	6.91	0.35

Number of organism per g

Treatment	Biopsy	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
C – Untreated Control	4	128	3	1000	0.217	1.77E+06	6.25	
	8	31	3	10000	0.206	4.51E+06	6.65	
	9	63	3	1000	0.204	9.26E+05	5.97	STDV
					Mean	2.40E+06	6.29	0.35

APPENDIX 5. Molecular Assessment raw data.

Well	Fluorophore	Target Name	Sample Name	Ct
A01	SYBR	Gapdh	D4-A7	22.99
A02	SYBR	Gapdh	D4-A7	23.05
A03	SYBR	Gapdh	D4-A7	22.99
A04	SYBR	IL-1	D4-A7	23.34
A05	SYBR	IL-1	D4-A7	23.26
A06	SYBR	IL-1	D4-A7	23.22
A07	SYBR	IL-6	D4-A7	27.30
A08	SYBR	IL-6	D4-A7	27.40
A09	SYBR	IL-6	D4-A7	27.28
A10	SYBR	MMP-1	D4-A7	18.59
A11	SYBR	MMP-1	D4-A7	18.95
A12	SYBR	MMP-1	D4-A7	18.60
B01	SYBR	Gapdh	D4-A8	22.97
B02	SYBR	Gapdh	D4-A8	23.07
B03	SYBR	Gapdh	D4-A8	23.07
B04	SYBR	IL-1	D4-A8	23.58
B05	SYBR	IL-1	D4-A8	23.60
B06	SYBR	IL-1	D4-A8	23.66
B07	SYBR	IL-6	D4-A8	26.65
B08	SYBR	IL-6	D4-A8	26.70
B09	SYBR	IL-6	D4-A8	26.73
B10	SYBR	MMP-1	D4-A8	19.06
B11	SYBR	MMP-1	D4-A8	19.06
B12	SYBR	MMP-1	D4-A8	19.15
C01	SYBR	Gapdh	D4-A9	23.51
C02	SYBR	Gapdh	D4-A9	23.64
C03	SYBR	Gapdh	D4-A9	23.70
C04	SYBR	IL-1	D4-A9	24.05
C05	SYBR	IL-1	D4-A9	24.29
C06	SYBR	IL-1	D4-A9	24.17
C07	SYBR	IL-6	D4-A9	28.87
C08	SYBR	IL-6	D4-A9	28.38
C09	SYBR	IL-6	D4-A9	28.32
C10	SYBR	MMP-1	D4-A9	20.91
C11	SYBR	MMP-1	D4-A9	21.02
C12	SYBR	MMP-1	D4-A9	21.04
D01	SYBR	Gapdh	D4-B7	22.73
D02	SYBR	Gapdh	D4-B7	22.64
D03	SYBR	Gapdh	D4-B7	22.38
D04	SYBR	IL-1	D4-B7	22.45
D05	SYBR	IL-1	D4-B7	22.49
D06	SYBR	IL-1	D4-B7	22.47
D07	SYBR	IL-6	D4-B7	26.72
D08	SYBR	IL-6	D4-B7	26.87
D09	SYBR	IL-6	D4-B7	26.68
D10	SYBR	MMP-1	D4-B7	19.30
D11	SYBR	MMP-1	D4-B7	19.22
D12	SYBR	MMP-1	D4-B7	19.43

Well	Fluorophore	Target Name	Sample Name	Ct
E01	SYBR	Gapdh	D4-B8	23.31
E02	SYBR	Gapdh	D4-B8	23.04
E03	SYBR	Gapdh	D4-B8	23.10
E04	SYBR	IL-1	D4-B8	23.64
E05	SYBR	IL-1	D4-B8	23.57
E06	SYBR	IL-1	D4-B8	23.58
E07	SYBR	IL-6	D4-B8	26.86
E08	SYBR	IL-6	D4-B8	26.59
E09	SYBR	IL-6	D4-B8	26.76
E10	SYBR	MMP-1	D4-B8	19.15
E11	SYBR	MMP-1	D4-B8	19.18
E12	SYBR	MMP-1	D4-B8	19.32
F01	SYBR	Gapdh	D4-B9	23.04
F02	SYBR	Gapdh	D4-B9	22.66
F03	SYBR	Gapdh	D4-B9	22.61
F04	SYBR	IL-1	D4-B9	23.14
F05	SYBR	IL-1	D4-B9	23.24
F06	SYBR	IL-1	D4-B9	23.30
F07	SYBR	IL-6	D4-B9	27.44
F08	SYBR	IL-6	D4-B9	27.37
F09	SYBR	IL-6	D4-B9	27.54
F10	SYBR	MMP-1	D4-B9	19.21
F11	SYBR	MMP-1	D4-B9	19.07
F12	SYBR	MMP-1	D4-B9	19.07
G01	SYBR	Gapdh	D4-C1	23.12
G02	SYBR	Gapdh	D4-C1	22.87
G03	SYBR	Gapdh	D4-C1	23.09
G04	SYBR	IL-1	D4-C1	24.85
G05	SYBR	IL-1	D4-C1	24.70
G06	SYBR	IL-1	D4-C1	24.81
G07	SYBR	IL-6	D4-C1	28.91
G08	SYBR	IL-6	D4-C1	28.81
G09	SYBR	IL-6	D4-C1	28.83
G10	SYBR	MMP-1	D4-C1	21.72
G11	SYBR	MMP-1	D4-C1	21.66
G12	SYBR	MMP-1	D4-C1	21.79
H01	SYBR	Gapdh	D4-C2	23.01
H02	SYBR	Gapdh	D4-C2	22.64
H03	SYBR	Gapdh	D4-C2	22.70
H04	SYBR	IL-1	D4-C2	23.31
H05	SYBR	IL-1	D4-C2	23.26
H06	SYBR	IL-1	D4-C2	23.24
H07	SYBR	IL-6	D4-C2	27.00
H08	SYBR	IL-6	D4-C2	26.93
H09	SYBR	IL-6	D4-C2	27.10
H10	SYBR	MMP-1	D4-C2	20.87
H11	SYBR	MMP-1	D4-C2	20.94
H12	SYBR	MMP-1	D4-C2	21.14

Well	Fluorophore	Target Name	Content	Sample Name	Ct
A01	SYBR	Gapdh	Unkn	D8-A1	25.01
A02	SYBR	Gapdh	Unkn	D8-A1	25.21
A03	SYBR	Gapdh	Unkn	D8-A1	25.07
A04	SYBR	IL-1	Unkn	D8-A1	26.07
A05	SYBR	IL-1	Unkn	D8-A1	26.02
A06	SYBR	IL-1	Unkn	D8-A1	25.89
A07	SYBR	IL-6	Unkn	D8-A1	30.16
A08	SYBR	IL-6	Unkn	D8-A1	30.28
A09	SYBR	IL-6	Unkn	D8-A1	29.98
A10	SYBR	MMP-1	Unkn	D8-A1	19.17
A11	SYBR	MMP-1	Unkn	D8-A1	19.19
A12	SYBR	MMP-1	Unkn	D8-A1	19.21
B01	SYBR	Gapdh	Unkn	D8-A2	25.16
B02	SYBR	Gapdh	Unkn	D8-A2	25.32
B03	SYBR	Gapdh	Unkn	D8-A2	25.29
B04	SYBR	IL-1	Unkn	D8-A2	26.36
B05	SYBR	IL-1	Unkn	D8-A2	26.21
B06	SYBR	IL-1	Unkn	D8-A2	26.15
B07	SYBR	IL-6	Unkn	D8-A2	31.74
B08	SYBR	IL-6	Unkn	D8-A2	31.49
B09	SYBR	IL-6	Unkn	D8-A2	32.02
B10	SYBR	MMP-1	Unkn	D8-A2	21.15
B11	SYBR	MMP-1	Unkn	D8-A2	21.12
B12	SYBR	MMP-1	Unkn	D8-A2	21.21
C01	SYBR	Gapdh	Unkn	D8-A3	25.01
C02	SYBR	Gapdh	Unkn	D8-A3	25.08
C03	SYBR	Gapdh	Unkn	D8-A3	25.34
C04	SYBR	IL-1	Unkn	D8-A3	24.67
C05	SYBR	IL-1	Unkn	D8-A3	24.78
C06	SYBR	IL-1	Unkn	D8-A3	24.61
C07	SYBR	IL-6	Unkn	D8-A3	30.20
C08	SYBR	IL-6	Unkn	D8-A3	29.50
C09	SYBR	IL-6	Unkn	D8-A3	29.49
C10	SYBR	MMP-1	Unkn	D8-A3	19.18
C11	SYBR	MMP-1	Unkn	D8-A3	19.20
C12	SYBR	MMP-1	Unkn	D8-A3	19.28
D01	SYBR	Gapdh	Unkn	D8-B1	26.59
D02	SYBR	Gapdh	Unkn	D8-B1	26.43
D03	SYBR	Gapdh	Unkn	D8-B1	26.66
D04	SYBR	IL-1	Unkn	D8-B1	25.46
D05	SYBR	IL-1	Unkn	D8-B1	25.34
D06	SYBR	IL-1	Unkn	D8-B1	25.36
D07	SYBR	IL-6	Unkn	D8-B1	31.53
D08	SYBR	IL-6	Unkn	D8-B1	31.18
D09	SYBR	IL-6	Unkn	D8-B1	31.52
D10	SYBR	MMP-1	Unkn	D8-B1	20.47
D11	SYBR	MMP-1	Unkn	D8-B1	20.53
D12	SYBR	MMP-1	Unkn	D8-B1	20.51

Well	Fluorophore	Target Name	Content	Sample Name	Ct
E01	SYBR	Gapdh	Unkn	D8-B2	25.36
E02	SYBR	Gapdh	Unkn	D8-B2	25.23
E03	SYBR	Gapdh	Unkn	D8-B2	25.25
E04	SYBR	IL-1	Unkn	D8-B2	24.37
E05	SYBR	IL-1	Unkn	D8-B2	24.13
E06	SYBR	IL-1	Unkn	D8-B2	24.33
E07	SYBR	IL-6	Unkn	D8-B2	30.94
E08	SYBR	IL-6	Unkn	D8-B2	30.91
E09	SYBR	IL-6	Unkn	D8-B2	30.55
E10	SYBR	MMP-1	Unkn	D8-B2	19.88
E11	SYBR	MMP-1	Unkn	D8-B2	19.88
E12	SYBR	MMP-1	Unkn	D8-B2	20.05
F01	SYBR	Gapdh	Unkn	D8-B3	N/A
F02	SYBR	Gapdh	Unkn	D8-B3	N/A
F03	SYBR	Gapdh	Unkn	D8-B3	N/A
F04	SYBR	IL-1	Unkn	D8-B3	N/A
F05	SYBR	IL-1	Unkn	D8-B3	N/A
F06	SYBR	IL-1	Unkn	D8-B3	N/A
F07	SYBR	IL-6	Unkn	D8-B3	N/A
F08	SYBR	IL-6	Unkn	D8-B3	N/A
F09	SYBR	IL-6	Unkn	D8-B3	N/A
F10	SYBR	MMP-1	Unkn	D8-B3	N/A
F11	SYBR	MMP-1	Unkn	D8-B3	37.07
F12	SYBR	MMP-1	Unkn	D8-B3	N/A
G01	SYBR	Gapdh	Unkn	D8-C5	24.98
G02	SYBR	Gapdh	Unkn	D8-C5	25.03
G03	SYBR	Gapdh	Unkn	D8-C5	25.02
G04	SYBR	IL-1	Unkn	D8-C5	25.16
G05	SYBR	IL-1	Unkn	D8-C5	24.81
G06	SYBR	IL-1	Unkn	D8-C5	24.93
G07	SYBR	IL-6	Unkn	D8-C5	32.01
G08	SYBR	IL-6	Unkn	D8-C5	31.36
G09	SYBR	IL-6	Unkn	D8-C5	31.41
G10	SYBR	MMP-1	Unkn	D8-C5	21.64
G11	SYBR	MMP-1	Unkn	D8-C5	21.49
G12	SYBR	MMP-1	Unkn	D8-C5	21.61
H01	SYBR	Gapdh	Unkn	D8-C6	26.57
H02	SYBR	Gapdh	Unkn	D8-C6	26.61
H03	SYBR	Gapdh	Unkn	D8-C6	26.83
H04	SYBR	IL-1	Unkn	D8-C6	27.00
H05	SYBR	IL-1	Unkn	D8-C6	26.90
H06	SYBR	IL-1	Unkn	D8-C6	27.04
H07	SYBR	IL-6	Unkn	D8-C6	32.09
H08	SYBR	IL-6	Unkn	D8-C6	32.04
H09	SYBR	IL-6	Unkn	D8-C6	32.30
H10	SYBR	MMP-1	Unkn	D8-C6	22.26
H11	SYBR	MMP-1	Unkn	D8-C6	22.29
H12	SYBR	MMP-1	Unkn	D8-C6	22.50

Note: The concentration and quality of RNA extracted from sample B3 on Day 8 were low. Real-time reactions did not work, likely due to poor RNA quality.

Well	Fluorophore	Target Name	Content	Sample Name	Ct
A01	SYBR	Gapdh	Unkn	D11-A1	25.44
A02	SYBR	Gapdh	Unkn	D11-A1	25.51
A03	SYBR	Gapdh	Unkn	D11-A1	25.46
A04	SYBR	IL-1	Unkn	D11-A1	28.59
A05	SYBR	IL-1	Unkn	D11-A1	28.64
A06	SYBR	IL-1	Unkn	D11-A1	28.51
A07	SYBR	IL-6	Unkn	D11-A1	32.21
A08	SYBR	IL-6	Unkn	D11-A1	32.15
A09	SYBR	IL-6	Unkn	D11-A1	32.09
A10	SYBR	MMP-1	Unkn	D11-A1	26.04
A11	SYBR	MMP-1	Unkn	D11-A1	26.04
A12	SYBR	MMP-1	Unkn	D11-A1	26.11
B01	SYBR	Gapdh	Unkn	D11-A2	24.84
B02	SYBR	Gapdh	Unkn	D11-A2	25.08
B03	SYBR	Gapdh	Unkn	D11-A2	25.01
B04	SYBR	IL-1	Unkn	D11-A2	27.16
B05	SYBR	IL-1	Unkn	D11-A2	27.12
B06	SYBR	IL-1	Unkn	D11-A2	27.12
B07	SYBR	IL-6	Unkn	D11-A2	31.74
B08	SYBR	IL-6	Unkn	D11-A2	31.67
B09	SYBR	IL-6	Unkn	D11-A2	32.02
B10	SYBR	MMP-1	Unkn	D11-A2	23.67
B11	SYBR	MMP-1	Unkn	D11-A2	23.72
B12	SYBR	MMP-1	Unkn	D11-A2	23.89
C01	SYBR	Gapdh	Unkn	D11-A3	25.89
C02	SYBR	Gapdh	Unkn	D11-A3	26.06
C03	SYBR	Gapdh	Unkn	D11-A3	26.31
C04	SYBR	IL-1	Unkn	D11-A3	27.01
C05	SYBR	IL-1	Unkn	D11-A3	27.08
C06	SYBR	IL-1	Unkn	D11-A3	27.02
C07	SYBR	IL-6	Unkn	D11-A3	33.86
C08	SYBR	IL-6	Unkn	D11-A3	33.57
C09	SYBR	IL-6	Unkn	D11-A3	33.55
C10	SYBR	MMP-1	Unkn	D11-A3	26.04
C11	SYBR	MMP-1	Unkn	D11-A3	25.97
C12	SYBR	MMP-1	Unkn	D11-A3	26.06
D01	SYBR	Gapdh	Unkn	D11-B1	26.32
D02	SYBR	Gapdh	Unkn	D11-B1	26.32
D03	SYBR	Gapdh	Unkn	D11-B1	26.20
D04	SYBR	IL-1	Unkn	D11-B1	28.49
D05	SYBR	IL-1	Unkn	D11-B1	28.63
D06	SYBR	IL-1	Unkn	D11-B1	28.44
D07	SYBR	IL-6	Unkn	D11-B1	32.03
D08	SYBR	IL-6	Unkn	D11-B1	32.63
D09	SYBR	IL-6	Unkn	D11-B1	31.93
D10	SYBR	MMP-1	Unkn	D11-B1	22.27
D11	SYBR	MMP-1	Unkn	D11-B1	22.30
D12	SYBR	MMP-1	Unkn	D11-B1	22.38

Well	Fluorophore	Target Name	Content	Sample Name	Ct
E01	SYBR	Gapdh	Unkn	D11-B2	27.25
E02	SYBR	Gapdh	Unkn	D11-B2	27.24
E03	SYBR	Gapdh	Unkn	D11-B2	27.18
E04	SYBR	IL-1	Unkn	D11-B2	27.14
E05	SYBR	IL-1	Unkn	D11-B2	27.15
E06	SYBR	IL-1	Unkn	D11-B2	27.09
E07	SYBR	IL-6	Unkn	D11-B2	34.14
E08	SYBR	IL-6	Unkn	D11-B2	33.49
E09	SYBR	IL-6	Unkn	D11-B2	33.83
E10	SYBR	MMP-1	Unkn	D11-B2	25.27
E11	SYBR	MMP-1	Unkn	D11-B2	25.19
E12	SYBR	MMP-1	Unkn	D11-B2	25.36
F01	SYBR	Gapdh	Unkn	D11-B3	24.98
F02	SYBR	Gapdh	Unkn	D11-B3	24.66
F03	SYBR	Gapdh	Unkn	D11-B3	24.74
F04	SYBR	IL-1	Unkn	D11-B3	26.73
F05	SYBR	IL-1	Unkn	D11-B3	26.58
F06	SYBR	IL-1	Unkn	D11-B3	26.57
F07	SYBR	IL-6	Unkn	D11-B3	30.13
F08	SYBR	IL-6	Unkn	D11-B3	30.09
F09	SYBR	IL-6	Unkn	D11-B3	30.24
F10	SYBR	MMP-1	Unkn	D11-B3	20.08
F11	SYBR	MMP-1	Unkn	D11-B3	19.91
F12	SYBR	MMP-1	Unkn	D11-B3	20.07
G01	SYBR	Gapdh	Unkn	D11-C1	25.76
G02	SYBR	Gapdh	Unkn	D11-C1	25.84
G03	SYBR	Gapdh	Unkn	D11-C1	25.53
G04	SYBR	IL-1	Unkn	D11-C1	27.00
G05	SYBR	IL-1	Unkn	D11-C1	26.84
G06	SYBR	IL-1	Unkn	D11-C1	26.82
G07	SYBR	IL-6	Unkn	D11-C1	32.61
G08	SYBR	IL-6	Unkn	D11-C1	32.22
G09	SYBR	IL-6	Unkn	D11-C1	32.55
G10	SYBR	MMP-1	Unkn	D11-C1	23.43
G11	SYBR	MMP-1	Unkn	D11-C1	23.40
G12	SYBR	MMP-1	Unkn	D11-C1	23.53
H01	SYBR	Gapdh	Unkn	D11-C2	25.17
H02	SYBR	Gapdh	Unkn	D11-C2	25.14
H03	SYBR	Gapdh	Unkn	D11-C2	25.62
H04	SYBR	IL-1	Unkn	D11-C2	27.28
H05	SYBR	IL-1	Unkn	D11-C2	27.28
H06	SYBR	IL-1	Unkn	D11-C2	27.26
H07	SYBR	IL-6	Unkn	D11-C2	30.76
H08	SYBR	IL-6	Unkn	D11-C2	30.73
H09	SYBR	IL-6	Unkn	D11-C2	30.84
H10	SYBR	MMP-1	Unkn	D11-C2	23.65
H11	SYBR	MMP-1	Unkn	D11-C2	23.70
H12	SYBR	MMP-1	Unkn	D11-C2	23.85

Well	Fluorophore	Target Name	Content	Sample Name	Ct
F01	SYBR	Gapdh	Unkn	D0-BL1	22.44
F02	SYBR	Gapdh	Unkn	D0-BL1	22.35
F03	SYBR	Gapdh	Unkn	D0-BL1	22.35
F04	SYBR	IL-1	Unkn	D0-BL1	22.61
F05	SYBR	IL-1	Unkn	D0-BL1	22.57
F06	SYBR	IL-1	Unkn	D0-BL1	22.72
F07	SYBR	IL-6	Unkn	D0-BL1	27.42
F08	SYBR	IL-6	Unkn	D0-BL1	27.49
F09	SYBR	IL-6	Unkn	D0-BL1	27.43
F10	SYBR	MMP-1	Unkn	D0-BL1	19.58
F11	SYBR	MMP-1	Unkn	D0-BL1	19.56
F12	SYBR	MMP-1	Unkn	D0-BL1	19.66
G01	SYBR	Gapdh	Unkn	D0-BL2	22.99
G02	SYBR	Gapdh	Unkn	D0-BL2	22.98
G03	SYBR	Gapdh	Unkn	D0-BL2	22.98
G04	SYBR	IL-1	Unkn	D0-BL2	22.84
G05	SYBR	IL-1	Unkn	D0-BL2	22.51
G06	SYBR	IL-1	Unkn	D0-BL2	22.64
G07	SYBR	IL-6	Unkn	D0-BL2	29.04
G08	SYBR	IL-6	Unkn	D0-BL2	28.57
G09	SYBR	IL-6	Unkn	D0-BL2	28.82
G10	SYBR	MMP-1	Unkn	D0-BL2	23.27
G11	SYBR	MMP-1	Unkn	D0-BL2	23.19
G12	SYBR	MMP-1	Unkn	D0-BL2	23.43
H01	SYBR	Gapdh	Unkn	D0-BL3	22.59
H02	SYBR	Gapdh	Unkn	D0-BL3	22.68
H03	SYBR	Gapdh	Unkn	D0-BL3	22.68
H04	SYBR	IL-1	Unkn	D0-BL3	23.01
H05	SYBR	IL-1	Unkn	D0-BL3	23.18
H06	SYBR	IL-1	Unkn	D0-BL3	23.05
H07	SYBR	IL-6	Unkn	D0-BL3	26.04
H08	SYBR	IL-6	Unkn	D0-BL3	26.13
H09	SYBR	IL-6	Unkn	D0-BL3	26.10
H10	SYBR	MMP-1	Unkn	D0-BL3	22.00
H11	SYBR	MMP-1	Unkn	D0-BL3	22.05
H12	SYBR	MMP-1	Unkn	D0-BL3	22.19

Well	Fluorophore	Target Name	Content	Sample Name	Ct
C01	SYBR	Gapdh	Unkn	D4-C3	24.88
C02	SYBR	Gapdh	Unkn	D4-C3	24.94
C03	SYBR	Gapdh	Unkn	D4-C3	25.22
C04	SYBR	IL-1	Unkn	D4-C3	24.15
C05	SYBR	IL-1	Unkn	D4-C3	24.11
C06	SYBR	IL-1	Unkn	D4-C3	24.17
C07	SYBR	IL-6	Unkn	D4-C3	29.63
C08	SYBR	IL-6	Unkn	D4-C3	29.32
C09	SYBR	IL-6	Unkn	D4-C3	29.30
C10	SYBR	MMP-1	Unkn	D4-C3	19.88
C11	SYBR	MMP-1	Unkn	D4-C3	20.04
C12	SYBR	MMP-1	Unkn	D4-C3	20.02
D01	SYBR	Gapdh	Unkn	D8-C7	27.74
D02	SYBR	Gapdh	Unkn	D8-C7	27.54
D03	SYBR	Gapdh	Unkn	D8-C7	27.59
D04	SYBR	IL-1	Unkn	D8-C7	27.27
D05	SYBR	IL-1	Unkn	D8-C7	27.20
D06	SYBR	IL-1	Unkn	D8-C7	27.22
D07	SYBR	IL-6	Unkn	D8-C7	31.12
D08	SYBR	IL-6	Unkn	D8-C7	31.58
D09	SYBR	IL-6	Unkn	D8-C7	31.54
D10	SYBR	MMP-1	Unkn	D8-C7	21.62
D11	SYBR	MMP-1	Unkn	D8-C7	21.59
D12	SYBR	MMP-1	Unkn	D8-C7	21.70
E01	SYBR	Gapdh	Unkn	D11-C3	25.94
E02	SYBR	Gapdh	Unkn	D11-C3	25.80
E03	SYBR	Gapdh	Unkn	D11-C3	25.91
E04	SYBR	IL-1	Unkn	D11-C3	25.73
E05	SYBR	IL-1	Unkn	D11-C3	25.72
E06	SYBR	IL-1	Unkn	D11-C3	25.79
E07	SYBR	IL-6	Unkn	D11-C3	31.70
E08	SYBR	IL-6	Unkn	D11-C3	31.60
E09	SYBR	IL-6	Unkn	D11-C3	31.85
E10	SYBR	MMP-1	Unkn	D11-C3	24.14
E11	SYBR	MMP-1	Unkn	D11-C3	24.09
E12	SYBR	MMP-1	Unkn	D11-C3	24.22

Well	Fluorophore	Target Name	Content	Sample Name	Ct
A01	SYBR	Gapdh	Unkn	D4-A7	23.91
A02	SYBR	Gapdh	Unkn	D4-A7	24.03
A03	SYBR	Gapdh	Unkn	D4-A7	23.87
A04	SYBR	MMP-9 F3-R3	Unkn	D4-A7	22.96
A05	SYBR	MMP-9 F3-R3	Unkn	D4-A7	23.01
A06	SYBR	MMP-9 F3-R3	Unkn	D4-A7	22.98
A07	SYBR	TNFa F2-R2	Unkn	D4-A7	27.38
A08	SYBR	TNFa F2-R2	Unkn	D4-A7	27.55
A09	SYBR	TNFa F2-R2	Unkn	D4-A7	27.22
B01	SYBR	Gapdh	Unkn	D4-A8	23.79
B02	SYBR	Gapdh	Unkn	D4-A8	23.91
B03	SYBR	Gapdh	Unkn	D4-A8	23.65
B04	SYBR	MMP-9 F3-R3	Unkn	D4-A8	24.28
B05	SYBR	MMP-9 F3-R3	Unkn	D4-A8	24.05
B06	SYBR	MMP-9 F3-R3	Unkn	D4-A8	23.92
B07	SYBR	TNFa F2-R2	Unkn	D4-A8	27.75
B08	SYBR	TNFa F2-R2	Unkn	D4-A8	27.70
B09	SYBR	TNFa F2-R2	Unkn	D4-A8	28.02
C01	SYBR	Gapdh	Unkn	D4-A9	23.76
C02	SYBR	Gapdh	Unkn	D4-A9	24.39
C03	SYBR	Gapdh	Unkn	D4-A9	25.12
C04	SYBR	MMP-9 F3-R3	Unkn	D4-A9	24.55
C05	SYBR	MMP-9 F3-R3	Unkn	D4-A9	24.47
C06	SYBR	MMP-9 F3-R3	Unkn	D4-A9	24.48
C07	SYBR	TNFa F2-R2	Unkn	D4-A9	28.13
C08	SYBR	TNFa F2-R2	Unkn	D4-A9	28.23
C09	SYBR	TNFa F2-R2	Unkn	D4-A9	28.04
D01	SYBR	Gapdh	Unkn	D4-B7	23.81
D02	SYBR	Gapdh	Unkn	D4-B7	24.21
D03	SYBR	Gapdh	Unkn	D4-B7	24.26
D04	SYBR	MMP-9 F3-R3	Unkn	D4-B7	23.22
D05	SYBR	MMP-9 F3-R3	Unkn	D4-B7	23.35
D06	SYBR	MMP-9 F3-R3	Unkn	D4-B7	23.16
D07	SYBR	TNFa F2-R2	Unkn	D4-B7	28.44
D08	SYBR	TNFa F2-R2	Unkn	D4-B7	28.43
D09	SYBR	TNFa F2-R2	Unkn	D4-B7	28.67

Well	Fluorophore	Target Name	Content	Sample Name	Ct
E01	SYBR	Gapdh	Unkn	D4-B8	25.07
E02	SYBR	Gapdh	Unkn	D4-B8	24.56
E03	SYBR	Gapdh	Unkn	D4-B8	N/A
E04	SYBR	MMP-9 F3-R3	Unkn	D4-B8	23.58
E05	SYBR	MMP-9 F3-R3	Unkn	D4-B8	23.33
E06	SYBR	MMP-9 F3-R3	Unkn	D4-B8	23.25
E07	SYBR	TNFa F2-R2	Unkn	D4-B8	29.54
E08	SYBR	TNFa F2-R2	Unkn	D4-B8	29.35
E09	SYBR	TNFa F2-R2	Unkn	D4-B8	29.42
F01	SYBR	Gapdh	Unkn	D4-B9	24.13
F02	SYBR	Gapdh	Unkn	D4-B9	24.16
F03	SYBR	Gapdh	Unkn	D4-B9	24.18
F04	SYBR	MMP-9 F3-R3	Unkn	D4-B9	22.55
F05	SYBR	MMP-9 F3-R3	Unkn	D4-B9	22.65
F06	SYBR	MMP-9 F3-R3	Unkn	D4-B9	22.44
F07	SYBR	TNFa F2-R2	Unkn	D4-B9	29.17
F08	SYBR	TNFa F2-R2	Unkn	D4-B9	29.30
F09	SYBR	TNFa F2-R2	Unkn	D4-B9	29.58
G01	SYBR	Gapdh	Unkn	D4-C1	24.66
G02	SYBR	Gapdh	Unkn	D4-C1	25.18
G03	SYBR	Gapdh	Unkn	D4-C1	24.53
G04	SYBR	MMP-9 F3-R3	Unkn	D4-C1	22.11
G05	SYBR	MMP-9 F3-R3	Unkn	D4-C1	22.10
G06	SYBR	MMP-9 F3-R3	Unkn	D4-C1	22.09
G07	SYBR	TNFa F2-R2	Unkn	D4-C1	30.90
G08	SYBR	TNFa F2-R2	Unkn	D4-C1	30.10
G09	SYBR	TNFa F2-R2	Unkn	D4-C1	30.16
H01	SYBR	Gapdh	Unkn	D4-C2	24.34
H02	SYBR	Gapdh	Unkn	D4-C2	25.03
H03	SYBR	Gapdh	Unkn	D4-C2	24.32
H04	SYBR	MMP-9 F3-R3	Unkn	D4-C2	23.03
H05	SYBR	MMP-9 F3-R3	Unkn	D4-C2	22.66
H06	SYBR	MMP-9 F3-R3	Unkn	D4-C2	23.04
H07	SYBR	TNFa F2-R2	Unkn	D4-C2	29.83
H08	SYBR	TNFa F2-R2	Unkn	D4-C2	29.86
H09	SYBR	TNFa F2-R2	Unkn	D4-C2	30.13

Well	Fluorophore	Target Name	Content	Sample Name	Ct
A01	SYBR	Gapdh	Unkn	D8-A1	24.11
A02	SYBR	Gapdh	Unkn	D8-A1	24.20
A03	SYBR	Gapdh	Unkn	D8-A1	23.97
A04	SYBR	MMP-9 F3-R3	Unkn	D8-A1	22.72
A05	SYBR	MMP-9 F3-R3	Unkn	D8-A1	22.48
A06	SYBR	MMP-9 F3-R3	Unkn	D8-A1	22.58
A07	SYBR	TNFa F2-R2	Unkn	D8-A1	32.88
A08	SYBR	TNFa F2-R2	Unkn	D8-A1	33.42
A09	SYBR	TNFa F2-R2	Unkn	D8-A1	33.32
B01	SYBR	Gapdh	Unkn	D8-A2	24.17
B02	SYBR	Gapdh	Unkn	D8-A2	24.02
B03	SYBR	Gapdh	Unkn	D8-A2	24.15
B04	SYBR	MMP-9 F3-R3	Unkn	D8-A2	23.06
B05	SYBR	MMP-9 F3-R3	Unkn	D8-A2	22.97
B06	SYBR	MMP-9 F3-R3	Unkn	D8-A2	23.03
B07	SYBR	TNFa F2-R2	Unkn	D8-A2	33.58
B08	SYBR	TNFa F2-R2	Unkn	D8-A2	34.04
B09	SYBR	TNFa F2-R2	Unkn	D8-A2	33.49
C01	SYBR	Gapdh	Unkn	D8-A3	23.99
C02	SYBR	Gapdh	Unkn	D8-A3	24.21
C03	SYBR	Gapdh	Unkn	D8-A3	24.34
C04	SYBR	MMP-9 F3-R3	Unkn	D8-A3	22.00
C05	SYBR	MMP-9 F3-R3	Unkn	D8-A3	22.27
C06	SYBR	MMP-9 F3-R3	Unkn	D8-A3	21.96
C07	SYBR	TNFa F2-R2	Unkn	D8-A3	33.07
C08	SYBR	TNFa F2-R2	Unkn	D8-A3	32.91
C09	SYBR	TNFa F2-R2	Unkn	D8-A3	32.79
D01	SYBR	Gapdh	Unkn	D8-B1	25.06
D02	SYBR	Gapdh	Unkn	D8-B1	25.01
D03	SYBR	Gapdh	Unkn	D8-B1	25.01
D04	SYBR	MMP-9 F3-R3	Unkn	D8-B1	22.78
D05	SYBR	MMP-9 F3-R3	Unkn	D8-B1	22.93
D06	SYBR	MMP-9 F3-R3	Unkn	D8-B1	22.76
D07	SYBR	TNFa F2-R2	Unkn	D8-B1	32.77
D08	SYBR	TNFa F2-R2	Unkn	D8-B1	32.64
D09	SYBR	TNFa F2-R2	Unkn	D8-B1	32.39

Well	Fluorophore	Target Name	Content	Sample Name	Ct
E01	SYBR	Gapdh	Unkn	D8-B2	24.80
E02	SYBR	Gapdh	Unkn	D8-B2	24.58
E03	SYBR	Gapdh	Unkn	D8-B2	24.79
E04	SYBR	MMP-9 F3-R3	Unkn	D8-B2	22.32
E05	SYBR	MMP-9 F3-R3	Unkn	D8-B2	22.21
E06	SYBR	MMP-9 F3-R3	Unkn	D8-B2	22.41
E07	SYBR	TNFa F2-R2	Unkn	D8-B2	32.38
E08	SYBR	TNFa F2-R2	Unkn	D8-B2	32.42
E09	SYBR	TNFa F2-R2	Unkn	D8-B2	32.27
F01	SYBR	Gapdh	Unkn	D8-B3	N/A
F02	SYBR	Gapdh	Unkn	D8-B3	N/A
F03	SYBR	Gapdh	Unkn	D8-B3	N/A
F04	SYBR	MMP-9 F3-R3	Unkn	D8-B3	N/A
F05	SYBR	MMP-9 F3-R3	Unkn	D8-B3	N/A
F06	SYBR	MMP-9 F3-R3	Unkn	D8-B3	39.45
F07	SYBR	TNFa F2-R2	Unkn	D8-B3	38.55
F08	SYBR	TNFa F2-R2	Unkn	D8-B3	39.53
F09	SYBR	TNFa F2-R2	Unkn	D8-B3	39.06
G01	SYBR	Gapdh	Unkn	D8-C5	24.27
G02	SYBR	Gapdh	Unkn	D8-C5	24.23
G03	SYBR	Gapdh	Unkn	D8-C5	24.01
G04	SYBR	MMP-9 F3-R3	Unkn	D8-C5	22.81
G05	SYBR	MMP-9 F3-R3	Unkn	D8-C5	22.65
G06	SYBR	MMP-9 F3-R3	Unkn	D8-C5	22.4
G07	SYBR	TNFa F2-R2	Unkn	D8-C5	33.95
G08	SYBR	TNFa F2-R2	Unkn	D8-C5	33.12
G09	SYBR	TNFa F2-R2	Unkn	D8-C5	34.07
H01	SYBR	Gapdh	Unkn	D8-C6	26.87
H02	SYBR	Gapdh	Unkn	D8-C6	26.33
H03	SYBR	Gapdh	Unkn	D8-C6	26.09
H04	SYBR	MMP-9 F3-R3	Unkn	D8-C6	24.84
H05	SYBR	MMP-9 F3-R3	Unkn	D8-C6	24.64
H06	SYBR	MMP-9 F3-R3	Unkn	D8-C6	24.71
H07	SYBR	TNFa F2-R2	Unkn	D8-C6	34.51
H08	SYBR	TNFa F2-R2	Unkn	D8-C6	34.29
H09	SYBR	TNFa F2-R2	Unkn	D8-C6	34.36

Well	Fluorophore	Target Name	Content	Sample Name	Ct
A01	SYBR	Gapdh	Unkn	D11-A1	25.17
A02	SYBR	Gapdh	Unkn	D11-A1	25.31
A03	SYBR	Gapdh	Unkn	D11-A1	25.40
A04	SYBR	MMP-9 F3-R3	Unkn	D11-A1	26.03
A05	SYBR	MMP-9 F3-R3	Unkn	D11-A1	25.91
A06	SYBR	MMP-9 F3-R3	Unkn	D11-A1	26.13
A07	SYBR	TNFa F2-R2	Unkn	D11-A1	35.47
A08	SYBR	TNFa F2-R2	Unkn	D11-A1	35.29
A09	SYBR	TNFa F2-R2	Unkn	D11-A1	34.94
B01	SYBR	Gapdh	Unkn	D11-A2	24.74
B02	SYBR	Gapdh	Unkn	D11-A2	24.52
B03	SYBR	Gapdh	Unkn	D11-A2	23.39
B04	SYBR	MMP-9 F3-R3	Unkn	D11-A2	24.55
B05	SYBR	MMP-9 F3-R3	Unkn	D11-A2	24.44
B06	SYBR	MMP-9 F3-R3	Unkn	D11-A2	24.29
B07	SYBR	TNFa F2-R2	Unkn	D11-A2	34.21
B08	SYBR	TNFa F2-R2	Unkn	D11-A2	34.44
B09	SYBR	TNFa F2-R2	Unkn	D11-A2	34.51
C01	SYBR	Gapdh	Unkn	D11-A3	24.84
C02	SYBR	Gapdh	Unkn	D11-A3	24.67
C03	SYBR	Gapdh	Unkn	D11-A3	25.08
C04	SYBR	MMP-9 F3-R3	Unkn	D11-A3	25.33
C05	SYBR	MMP-9 F3-R3	Unkn	D11-A3	25.56
C06	SYBR	MMP-9 F3-R3	Unkn	D11-A3	25.37
C07	SYBR	TNFa F2-R2	Unkn	D11-A3	34.67
C08	SYBR	TNFa F2-R2	Unkn	D11-A3	34.18
C09	SYBR	TNFa F2-R2	Unkn	D11-A3	35.03
D01	SYBR	Gapdh	Unkn	D11-B1	25.78
D02	SYBR	Gapdh	Unkn	D11-B1	25.62
D03	SYBR	Gapdh	Unkn	D11-B1	25.62
D04	SYBR	MMP-9 F3-R3	Unkn	D11-B1	25.30
D05	SYBR	MMP-9 F3-R3	Unkn	D11-B1	25.24
D06	SYBR	MMP-9 F3-R3	Unkn	D11-B1	25.05
D07	SYBR	TNFa F2-R2	Unkn	D11-B1	36.22
D08	SYBR	TNFa F2-R2	Unkn	D11-B1	36.15
D09	SYBR	TNFa F2-R2	Unkn	D11-B1	37.72

Well	Fluorophore	Target Name	Content	Sample Name	Ct
E01	SYBR	Gapdh	Unkn	D11-B2	27.17
E02	SYBR	Gapdh	Unkn	D11-B2	26.58
E03	SYBR	Gapdh	Unkn	D11-B2	26.58
E04	SYBR	MMP-9 F3-R3	Unkn	D11-B2	25.48
E05	SYBR	MMP-9 F3-R3	Unkn	D11-B2	25.46
E06	SYBR	MMP-9 F3-R3	Unkn	D11-B2	25.49
E07	SYBR	TNFa F2-R2	Unkn	D11-B2	34.51
E08	SYBR	TNFa F2-R2	Unkn	D11-B2	34.69
E09	SYBR	TNFa F2-R2	Unkn	D11-B2	35.31
F01	SYBR	Gapdh	Unkn	D11-B3	24.57
F02	SYBR	Gapdh	Unkn	D11-B3	24.09
F03	SYBR	Gapdh	Unkn	D11-B3	24.16
F04	SYBR	MMP-9 F3-R3	Unkn	D11-B3	23.72
F05	SYBR	MMP-9 F3-R3	Unkn	D11-B3	23.47
F06	SYBR	MMP-9 F3-R3	Unkn	D11-B3	23.67
F07	SYBR	TNFa F2-R2	Unkn	D11-B3	34.25
F08	SYBR	TNFa F2-R2	Unkn	D11-B3	33.91
F09	SYBR	TNFa F2-R2	Unkn	D11-B3	33.41
G01	SYBR	Gapdh	Unkn	D11-C1	24.46
G02	SYBR	Gapdh	Unkn	D11-C1	24.26
G03	SYBR	Gapdh	Unkn	D11-C1	24.35
G04	SYBR	MMP-9 F3-R3	Unkn	D11-C1	23.64
G05	SYBR	MMP-9 F3-R3	Unkn	D11-C1	23.41
G06	SYBR	MMP-9 F3-R3	Unkn	D11-C1	23.30
G07	SYBR	TNFa F2-R2	Unkn	D11-C1	34.04
G08	SYBR	TNFa F2-R2	Unkn	D11-C1	34.15
G09	SYBR	TNFa F2-R2	Unkn	D11-C1	34.01
H01	SYBR	Gapdh	Unkn	D11-C2	25.00
H02	SYBR	Gapdh	Unkn	D11-C2	24.32
H03	SYBR	Gapdh	Unkn	D11-C2	24.35
H04	SYBR	MMP-9 F3-R3	Unkn	D11-C2	23.28
H05	SYBR	MMP-9 F3-R3	Unkn	D11-C2	23.34
H06	SYBR	MMP-9 F3-R3	Unkn	D11-C2	23.21
H07	SYBR	TNFa F2-R2	Unkn	D11-C2	34.04
H08	SYBR	TNFa F2-R2	Unkn	D11-C2	34.69
H09	SYBR	TNFa F2-R2	Unkn	D11-C2	34.91

Well	Fluorophore	Target Name	Content	Sample Name	Ct
C01	SYBR	Gapdh	Unkn	D0-BL1	24.23
C02	SYBR	Gapdh	Unkn	D0-BL1	24.34
C03	SYBR	Gapdh	Unkn	D0-BL1	24.42
C04	SYBR	MMP-9 F3-R3	Unkn	D0-BL1	23.51
C05	SYBR	MMP-9 F3-R3	Unkn	D0-BL1	23.37
C06	SYBR	MMP-9 F3-R3	Unkn	D0-BL1	23.53
C07	SYBR	TNFA F2-R2	Unkn	D0-BL1	31.03
C08	SYBR	TNFA F2-R2	Unkn	D0-BL1	30.73
C09	SYBR	TNFA F2-R2	Unkn	D0-BL1	31.30
D01	SYBR	Gapdh	Unkn	D0-BL2	24.73
D02	SYBR	Gapdh	Unkn	D0-BL2	24.63
D03	SYBR	Gapdh	Unkn	D0-BL2	24.66
D04	SYBR	MMP-9 F3-R3	Unkn	D0-BL2	25.76
D05	SYBR	MMP-9 F3-R3	Unkn	D0-BL2	25.61
D06	SYBR	MMP-9 F3-R3	Unkn	D0-BL2	25.38
D07	SYBR	TNFA F2-R2	Unkn	D0-BL2	31.63
D08	SYBR	TNFA F2-R2	Unkn	D0-BL2	31.66
D09	SYBR	TNFA F2-R2	Unkn	D0-BL2	31.95
E01	SYBR	Gapdh	Unkn	D0-BL3	24.23
E02	SYBR	Gapdh	Unkn	D0-BL3	23.97
E03	SYBR	Gapdh	Unkn	D0-BL3	24.46
E04	SYBR	MMP-9 F3-R3	Unkn	D0-BL3	25.16
E05	SYBR	MMP-9 F3-R3	Unkn	D0-BL3	24.95
E06	SYBR	MMP-9 F3-R3	Unkn	D0-BL3	25.15
E07	SYBR	TNFA F2-R2	Unkn	D0-BL3	31.60
E08	SYBR	TNFA F2-R2	Unkn	D0-BL3	31.57
E09	SYBR	TNFA F2-R2	Unkn	D0-BL3	31.23

Well	Fluorophore	Target Name	Content	Sample Name	Ct
F01	SYBR	Gapdh	Unkn	D4-C3	25.20
F02	SYBR	Gapdh	Unkn	D4-C3	24.76
F03	SYBR	Gapdh	Unkn	D4-C3	25.03
F04	SYBR	MMP-9 F3-R3	Unkn	D4-C3	22.66
F05	SYBR	MMP-9 F3-R3	Unkn	D4-C3	22.82
F06	SYBR	MMP-9 F3-R3	Unkn	D4-C3	22.28
F07	SYBR	TNFa F2-R2	Unkn	D4-C3	29.53
F08	SYBR	TNFa F2-R2	Unkn	D4-C3	29.25
F09	SYBR	TNFa F2-R2	Unkn	D4-C3	29.14
G01	SYBR	Gapdh	Unkn	D8-C7	27.50
G02	SYBR	Gapdh	Unkn	D8-C7	27.53
G03	SYBR	Gapdh	Unkn	D8-C7	27.15
G04	SYBR	MMP-9 F3-R3	Unkn	D8-C7	24.09
G05	SYBR	MMP-9 F3-R3	Unkn	D8-C7	24.06
G06	SYBR	MMP-9 F3-R3	Unkn	D8-C7	23.68
G07	SYBR	TNFa F2-R2	Unkn	D8-C7	30.67
G08	SYBR	TNFa F2-R2	Unkn	D8-C7	30.62
G09	SYBR	TNFa F2-R2	Unkn	D8-C7	29.87
H01	SYBR	Gapdh	Unkn	D11-C3	26.48
H02	SYBR	Gapdh	Unkn	D11-C3	26.57
H03	SYBR	Gapdh	Unkn	D11-C3	26.41
H04	SYBR	MMP-9 F3-R3	Unkn	D11-C3	24.84
H05	SYBR	MMP-9 F3-R3	Unkn	D11-C3	24.82
H06	SYBR	MMP-9 F3-R3	Unkn	D11-C3	24.78
H07	SYBR	TNFa F2-R2	Unkn	D11-C3	31.34
H08	SYBR	TNFa F2-R2	Unkn	D11-C3	31.39
H09	SYBR	TNFa F2-R2	Unkn	D11-C3	31.03

REFERENCES

[1] Sullivan TP, Eaglstein WH, Davis SC, and Mertz PM. The pig as a model for human wound healing. Wound Repair and Regeneration 9, 2, 2001, 66-76

[2] Davis SC, Ricotti C, Cazzaniga AL, Welch E, and Mertz PM. Microscopic and Physiological Evidencefor Biofilm-Associated Wound Colonization in-vivo *Wound Rep Reg* 2008 (16); 23–29

[3] Mertz PM, Alvarez OM, Smerbeck RV, Eaglstein WH: A new *in vivo* model for the evaluation of topical antiseptics on superficial wounds: The effect of 70% alcohol and povidone-iodine solution. *Arch. Dermatol*. 120:58-62, January 1984.

[4] Mertz PM, Marshall DA, Kuglar MA: Povidone iodine in polyethylene oxide hydrogel dressing. Effect on multiplication of *Staphylococcus aureus* in partial-thickness wounds. *Arch. Dermatol*. 112:1133-8, October 1986.

[5]Oliveria MF, Davis SC and Mertz PM: Can occlusive dressing composition influence proliferation of bacterial wound pathogens? *Wounds* 10(1):4-11,1998.

[6] Mertz PM, Oliverira-Gandia MF and Davis SC: The evaluation of a cadexomer iodine wound dressing on methicllin resistant staphylococcus aureus (MRSA) in acute wounds *Derm Surg 1999;25:89-93*

[7] Mertz, PM., Davis, SC, Cazzaniga, A., Drosou, A., Eaglstein, W. Barrier and Antibacterial Properties of 2-Octyl Cyanoacrylate Derived Wound Treatment Films *Journal of Cutaneous Medicine and Surgery 2003;7,1, 1-12.*

[8] Davis SC, Cazzaniga AL, Eaglstein WH, and Mertz PM. Over-The-Counter Antimicrobial Bandages and Proliferation of a Common Wound Pathogen. *Arch Dermatol Res.* 2005 Nov;297(5):190-5.

[9] Davis SC, Ricotti C, Cazzaniga AL, Welch E, and Mertz PM. Microscopic and Physiological Evidence for Biofilm-Associated Wound Colonization *In-vivo Wound Repair and Regen*: 2008, 16(1):23-9.

[10] Davis SC, Gil J, Solis M, Higa A, Mills A, Simms C, Valencia-Pena P, Li J, Raut V Antimicrobial Effectiveness of Wound Matrices containing Native Extra Cellular Matrix (ECM) with Polyhexamethylene Biguanide (PHMB), Int Wound J. 2021;1–14. DOI: 10.1111/iwj.13600

[11] Davis SC, Li J, Gil J, Valdes J, Solis M, Higa A, Bowler P. The Wound Healing Effects of a Next-Generation Anti-Biofilm Silver Hydrofiber® Wound Dressing on Deep Partial Thickness Wounds using a Porcine Model Int Wound J. 2018 Jun 11. doi: 10.1111/iwj.12935.